Subterranean clover to increase the legume content of pastures in spring

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Traditional ryegrass/white clover pastures frequently have less than the optimum 50% legume content for animal production, particularly in early spring when white clover growth rates are low. Three experiments were used to investigate ways to increase the legume content of pastures in early spring.

Experiments 1 & 2 investigated the potential for over-drilling subterranean clover in autumn into established ryegrass-based pastures to increase dry matter yields and legume content in early spring. Dry matter yields in Experiment 1 were 310 kg DM/ha greater in 'Woogenellup' pastures than controls, although these yields were depressed by higher than average rainfall and slug and grass grub damage. Total clover content in 'Woogenellup' reached 13.6% by early October, compared with 8.18% in the control. Experiment 2 at Invernia, a commercial dairy farm in North Otago, aimed to introduce subterranean clover under normal management of a dairy farm. Dry matter yields were not increased, but clover content was higher in 'Woogenellup' (21.7%) and 'Antas' (17.1%) than 'Denmark' (9.22%) and the control (5.95%). Subterranean clover was over-drilled into a second paddock, with no experimental design. In there, the subterranean clover content of pasture reached 25% in some areas, however it did not grow where white clover made up >25% of the pasture in spring. 'Woogenellup' showed greater production than 'Denmark' at both experiments, and 'Antas' was more productive in the higher pH soils of Experiment 2. The maximum subterranean clover yield measured in Experiments 1 & 2 was 320 kg DM/ha. Over-drilling subterranean clover was estimated to be profitable if yields could reach 500-1000 kg DM/ha.

Experiment 3 applied gibberellic acid (GA) to pure subterranean clover swards to increase spring dry matter yields. However, dry matter yields were not increased by GA at any stage in the experiment. Plant height did increase 44 days after GA application. In mixed pastures, this may lead to increased light interception of subterranean clover, and represents an area for further work. Large leaved cultivars produced more dry matter than small leaved cultivars, which was consistent with results at Experiments 1 & 2. Dry matter yields of 3.5-4 t DM/ha in 'Narrikup', 'Woogenellup' and 'Antas' were recorded in early October.

The concept of over-drilling subterranean clover into dairy pastures to increase early spring dry matter yields and production was successful, but not at levels that would support a financial return. The concept requires further work, which could include using balansa clover, which survives in the wet conditions that depressed subterranean clover growth in Experiments 1 & 2.

Keywords: *Trifolium subterraneum, Trifolium repens, Lolium perenne,* legume content, over-drilling, gibberellic acid, phyllochron, plant height.

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1 GENERAL INTRODUCTION

Traditional dairy pastures in New Zealand have been dominated by perennial ryegrass (*Lolium perenne*) / white clover (*Trifolium repens*) mixes (Da Silva *et al.,* 2004). The popularity is due to ease of establishment, tolerance of a wide range of environments, complementary growth patterns and high pasture production (Nobilly, 2015). However these pastures can be limited by poor persistence of white clover in areas with no or unreliable irrigation (Singh *et al.,* 1999). In addition, a decrease in nutritive value occurs in late spring for perennial ryegrass as more reproductive tillers are produced (Wims *et al.,* 2017). There are also slow growth rates of pastures at the beginning of the lactation period (McEvoy *et al.,* 2008). These limitations can be mitigated by the use of supplementary feeds, particularly early and late in the milking season when pasture growth rates are low. Grass silage is a common supplement used to balance the nutritive demand of dairy cattle with pasture production (Mackle *et al.,* 1999). However milk production has been shown to be greater when cows are fed on grazed pasture compared with grass silage (Dillon *et al.,* 2002). Therefore it is beneficial for farmers to find ways to increase pasture production to meet demand early in the season in place of feeding out grass silage.

The use of diverse pastures (pastures with three or more species) is a tool that is being promoted to the dairy industry. They have shown an ability to improve the nitrogen (N) use efficiency of dairy cows which could aid industry work towards environmental goals (Totty *et al.,* 2013). However reports have shown no increases in either dry matter intake or milk solid yield (Barry, 2015), which has led to limited farmer usage of these pastures. Increases in dry matter intake and metaboliseable energy (ME) intake have been shown to increase milk production (Mackle *et al.,* 1999). Furthermore, increases in the legume content of pasture has been shown to maximise milk yield (Cosgrove *et al.,* 2006). One way to increase early spring production and legume content of pastures could be to use winter annual legumes, and in this study the emphasis is on subterranean clover (*Trifolium subterraneum*). Subterranean clover has the potential to increase the dry matter and ME production of pastures in late winter/early spring due to its ability to produce high quality biomass during this period (Ates *et al.,* 2010).

Subterranean clover is a winter annual legume that produces its herbage in late winter and early spring, much earlier in the season compared with white clover (Mills *et al.,* 2014). In dryland systems subterranean clover sets seed in late spring/early summer, before moisture stress occurs. Emergence of seedlings occurs after the first autumn rains, allowing the plant to avoid summer droughts. Subterranean clover has been widely used in Australia and in parts of dryland New Zealand for sheep and beef production. To avoid losing moisture from the soil, subterranean clover is most commonly direct drilled into soil, usually as part of a pasture mix with other drought tolerant species (Murray, 2012). Over-drilling subterranean clover into irrigated dairy pastures is an area that has received no research, and it may not work. However, Ates *et al.,* (2010) showed increases in spring DM yield of 23-45% when subterranean clover was over-drilled into dryland cocksfoot (*Dactylis*)/ryegrass pastures, showing that it is relatively easy to introduce into cultivatable land that most dairy pastures are located on.

Gibberellic acid (GA) is a plant growth hormone that helps to promote stem elongation and plant growth (Lewak & Khan, 1977). Early studies have shown GA increased yields initially, followed by a lag phase where plant growth was decreased (Morgan & Mees, 1958). However, this lag has been reported to be due to excessive rates of application. More recent articles have stated application rates of 8 g GA/ha, compared with early studies that used rates of up to 700 g GA/ha (Matthew *et al.,* 2009). Using lower rates, GA application was shown to increase spring clover yields by 53% (van Rossum, 2013). This points to the potential for GA to be a useful tool for increasing pasture and clover yields during early lactation on dairy farms.

Recent popular press articles have reported on farmer efforts to increase legume content of pasture by over-drilling subterranean clover into permanent summer moist pasture in the Catlins (Cosgrove, 2016). However, there is no experimental data available to test whether over-drilling subterranean clover can be successful in a dairy pasture. Therefore, the aim of this study is to increase early spring growth and clover content of permanent ryegrass based irrigated or summer moist pastures. The specific objectives are: 1) to investigate and quantify the potential for over-drilling subterranean clover into irrigated or summer-moist established ryegrass pastures, and 2) to investigate and quantify the potential for the use of gibberellic acid to increase early spring growth of subterranean clover.

This dissertation is in six chapters. Chapter 2 is a literature review that covers the importance of legumes in dairy pastures, followed by the limitations of white clover being the sole legume of dairy pastures. Subterranean clover is then reviewed on its potential as an additional legume into dairy pastures, followed by methods that could be used to improve spring legume content such as over-drilling subterranean clover into pastures, and the use of gibberellic acid to increase spring growth of pasture. Chapter 3 details the materials and methods used to carry out three experiments. Experiment 1 and 2 explored whether over-drilling subterranean clover into permanent, moist ryegrass pastures could increase the early spring legume content of pastures. Experiment 1 was carried out at Lincoln University, New Zealand, while Experiment 2 was carried out at a commercial dairy farm in North Otago, New Zealand. Experiment 3 investigated the use of gibberellic acid to increase the production of pure subterranean clover swards at Lincoln University. Chapter 4 details the results these experiments, followed by their discussion in Chapter 5, and a general discussion and conclusions in Chapter 6.

2 REVIEW OF THE LITERATURE

2.1 Introduction.

Increasing the energy intake of dairy cows will result in an increase in milk solid production and therefore an increase in farm revenue (Dalley *et al.,* 2005). The two ways energy intake can be increased is by increasing feed quality or increasing feed supply. Increasing the clover content of pastures has been shown to increase the feed quality of pastures, and can result in a greater energy intake for grazing animals (Woodward *et al.,* 2003). Increasing legume content also adds fixed nitrogen back into pastures, which can decrease fertilizer costs for farmers. However, white clover is limited by the fact that its growing season doesn't start until October, meaning pastures are often low in legume content in early spring (Cooper *et al.,* 1997). Further, white clover is susceptible to attack from clover root weevil (*Sitona Lepidus*) (Crush *et al.,* 2007). These factors, combined with excessive sowing rates of companion grass species (Hurst *et al.,* 2000), make it difficult to increase white clover content of dairy pastures.

Subterranean clover has the potential to become an additional legume in dairy pastures. While there is no recent literature on its use in New Zealand pastures, the early spring growth of subterranean clover (Widdup & Pennel, 2000) could fit in well in dairy systems. This this review investigates the need for increasing clover content on dairy farms, and explain why this is difficult to do with white clover. Subterranean clover will be investigated as a potential additional legume for dairy pastures, along with any previous studies where GA or over-drilling has been used as a method for increasing clover content. The potential issues with these two methods will also be investigated. While all previous research in the literature on subterranean clover relates to dryland pastures, conclusions will be made in the context of irrigated dairy pastures to evaluate its possibility inclusion as an additional legume in dairy pastures.

2.2 Clover content in dairy pastures.

2.2.1 Current methods for meeting feed demand.

Using bought in feed such as maize (*Zea mays*)silage to meet feed demand and increase milk solid production can be unprofitable on dairy farms (Dalley *et al.,* 2005). This is because this type of farming system relies on two things; the cost of the bought in feed and the price per kilo of milk solids. If the cost of feed is high and the payout is low, economic farm surplus will decrease. Dalley *et al.,* (2005) compared the production and economic farm surplus between two different farm systems near Waimate. A stocking rate of 3.8 Jersey cows/ha with no bought in feed (LSR) was compared against a high stocking rate (5 cows/ha) using maize silage as supplementation (HSR) over four seasons. Maize silage was feed at up to 1.5 t DM/ha/year in the HSR system. Milk solid production per ha increased by 34% on the HSR farmlet (1566 kg MS/ha/yr) compared with the LSR farmlet (1172 kg MS/ha/yr). Milk solid response to the additional dry matter in the HSR system averaged 80 g MS/kg extra DM consumed. The economic farm surplus (EFS) was higher in the LSR system compared with the HSR system ($2784/ha compared with $2551/ha). This comparison showed the effectiveness of an increase in DM intake leading to an increase in milk solid production. However, the vulnerability of systems reliant on bought in feed was also highlighted by the range of EFS across years with different milk payout or supplement costs. Ideally farmers want to increase DM (or metaboliseable energy) intake without increasing feed costs.

Cows fed supplements had a higher substitution rate when grazing pure perennial ryegrass swards compared with ryegrass/ white clover pastures (Stockdale, 2000). Substitution rate is the decrease in pasture intake per kg of supplement fed (kg DM/ kg DM supplement). Substitution occurs when there is a decrease in rumen pH due to the rapid fermentation of supplements with a high nutritional value, in particular concentrates (Baudracco *et al.,* 2010). This leads to a reduction in the rate of fibre digestion and consequently decreased herbage intake. Pastures with higher clover content are more digestible, meaning pasture intake is less affected by a drop in rumen pH than pure ryegrass pastures. Stockdale (2000) reviewed data on factors that affect the substitution rate of cattle when fed supplements. They found that cows fed supplements whilst grazing pure ryegrass pastures had a supplementation rate 0.16 kg DM/kg DM of supplement greater than cows grazing ryegrass/white clover pastures. For New Zealand dairy systems, this points to a need for increased clover contents of pastures in early spring and autumn. These periods are when supplement feeding is the highest due to pasture growth rates not fully meeting animal demand.

2.2.2 Ryegrass/white clover pastures and feed demand of dairy cows.

Increases in the white clover content in pastures has led to increases in the milk solid production of dairy cows (Woodward *et al.,* 2003; Woodward *et al.,* 2001). This is because white clover has a higher nutritive value than perennial ryegrass, resulting in greater milk solid production per unit of feed intake. These are further amplified in summer when the nutritive value of perennial ryegrass drops due to the production of reproductive tillers. Woodward *et al.,* (2001) and Woodward *et al.,* (2003) summarised the same experiment near Hamilton. 'Old' and 'recent' cultivars of both perennial ryegrass and white clover were sown in pasture mixes. Data on milk production and economic farm surplus were recorded. Eerens *et al.,* (2001) summarised the botanical composition and pasture production from the same experiment. A pasture shown to have higher clover content ($P < 0.05$) by Eerens *et al.,* (2001) produced more milk solids per days in milk (1.42 kg/cow) compared with the other three pastures in the trial (1.38, 1.38 and 1.33 kg/cow, LSD = 0.03). These results were backed by conclusions drawn by Cosgrove *et al.,* (2006). They showed cows grazing pure ryegrass during the day and pure white clover at night had a milk solid yield of 2.0 kg MS/cow/day. This was higher ($P < 0.01$) than the 1.5 kg MS/cow/day for cows grazing pure ryegrass or a ryegrass/clover pasture with 8% clover.

The optimum white clover content of dairy pasture was shown to be 50-65% (Harris *et al.,* 1997). This range represents a balance between improving pasture quality, driven by the high metaboliseable energy content of clover, and maximising pasture yields, driven by the high growth rate of perennial ryegrass. Harris *et al.,* (1997) showed the effects on pasture intake, milk yield and milk composition of cows grazing pastures with a white clover content of 0%, 25%, 50% or 75% (Table 2.1). Cows were given two different feed allowances; restricted (25 kg DM/cow/day) or *ad lib* (50kg DM/cow/day). Pasture intake of cows grazing *ad lib* on 50% (14.8 kg DM/cow/d) and 75% (15.8 kg DM/cow/d) clover pasture was higher (P < 0.05) than cows grazing 0% (12.1 kg DM/cow/d) clover pasture. Under the *ad lib* feed allowance, cows grazing 25% clover pastures had a 22% increase in milk yield when compared with 0% clover pastures. Cows grazing 50% and 75% clover pastures had a 33% greater milk yield than 0% pastures. Under restricted allowances, milk yield was lower than fully fed cows; however responses to increases in clover content were similar in percentage terms. These results indicate that milk production continued to increase until clover content reached about 50%. Above this level, the decrease in dry matter production from increasing clover content leads to a decrease in energy intake of grazing animals. This suggests that an increase in clover content in early spring would lead to greater milk production.

Table 2.1: **Pasture intake, DM%, milk yield (L/cow/d) and milk solid production (kg MS/cow/d) of cows fed 0, 25, 50 and 75% clover under two different feed allocations (Harris *et al.,* 1997).**

Clover content	0%		25%		50%		75%	
Feed Allowance	*Ad lib*	Restricted	*Ad lib*	Restricted	*Ad lib*	Restricted	*Ad lib*	Restricted
Intake (kg DM/cow/d)	12.10	10.89	13.07	11.10	14.84	11.47	15.78	11.59
Milk yield (L/cow/d)	10.24	9.03	12.54	11.08	13.57	11.91	13.72	12.44
Milk solids (kg/cow/d)	0.96	0.84	1.17	1.01	1.24	1.09	1.26	1.10
Pasture DM %	21.0		16.4		14.9		15.0	

Advances in plant breeding have led to increases in the clover content of perennial ryegrass/white clover pastures (Woodward *et al.,* 2003). This is due to plant breeders focusing on improving the stolon density, leaf area and cool season growth of new cultivars. Cooper *et al.,* (1997) summarised the characteristics of the white clover cultivar 'Challenge', the most recent cultivar at the time of publication. They noted increased stolon densities and cool season growth made 'Challenge' suitable for rotationally grazed pastures. 'Challenge' white clover was used in the trial summarised by Woodward *et al.,* (2003). They compared the production and economic farm surplus on farmlets using either 'recent' or 'old' ryegrass and white clover cultivars. Pastures sown with recent white clover cultivars ('Challenge' and 'Sustain') had increased clover content compared with 'old' white clover cultivars. For example, in the 2001/02 season, 'recent' white clover cultivars sown with either 'recent' or 'old' perennial ryegrass cultivars had clover contents of 27.4% and 29.0%, respectively. In comparison, white clover cultivars from the 1960s with 'recent' and 'old' ryegrass cultivars had clover contents of 15.3% and 21.2% respectively (LSD = 4.3%). Of note is the fact that none of these pastures reached the 50% clover content that Harris *et al.,* (1997) noted as optimal for milk production. This is likely due to excessive rates of perennial ryegrass being used in pasture mixes, resulting in too much competition and consequently poor establishment of clover species (Hurst *et al.,* 2000).

Interestingly, the use of 'new' or 'old' ryegrass showed no difference in clover content when used with the 'new' clover. This highlights the slow genetic gains made by plant breeders with ryegrasses (Woodward *et al.,* 2003). Increases in ryegrass yields did not occur until the introduction of novel endophytes. These endophytes increased yields by decreasing the insect pressure on perennial ryegrass from pests such as Argentine stem weevil (*Listronotus bonariensis*) (ASW), which resulted in greater persistence and therefore increased yields (Milne, 2007).

Large leaved clover cultivars were shown to be more productive than small leaved cultivars in rotationally grazed ryegrass pastures. This is because large leaved cultivars have a greater ability to elongate petioles, meaning leaves were placed above the ryegrass canopy. This allows the clover to intercept more light, resulting in greater growth. Brock (1988) showed the dry matter production under set stocking and rotational grazing of four white clover cultivars with different leaf sizes. ‘Kopu’ had a leaf area of 5.57 cm^2, compared with 2.75 cm^2 for ‘Huia’. ‘Kopu’ produced 590 kg DM/ha under rotational grazing, which was 31% more than ‘Huia’. This information shows that large leaved clover cultivars will probably be more productive in rotationally grazed dairy pastures than small leaved cultivars.

2.2.3 Legumes as a tool for keeping production costs low.

Increasing clover contents of pastures can help maximise gross margins and increase milk solid production on dairy farms (Clark & Harris, 1996). Increasing clover content allows for lower nitrogen fertilizer inputs due to nitrogen fixation. Further, the higher nutritive value of white clover increases the quality of the pasture. This can bring increases in milk solid production if pasture yields can be kept constant while increasing clover content. Clark & Harris (1996) used UDDER, a dairy farm simulation model, to find the optimum combination of clover content and nitrogen fertilizer use. They noted a 50% clover pasture would need to produce 14.2 t DM/ha/year to generate the same gross margin as a 20% clover pasture yielding 16.2 t DM/ha/year. They noted an optimum system to include clover contents of 40% and nitrogen fertilizer inputs of 100-200 kg N/ha/year. This is lower than the optimum levels of 50-65% noted by Harris *et al.,* (1997). The reason for the slightly higher optimum clover content suggested by Harris *et al.,* (1997) was possibly due to differences in stocking rate or nitrogen fertilizer application rates between the two trials. The optimums stated in both papers are considerably higher than the clover contents estimated by Harris *et al.,* (1997) of 10-20% in most pastures. This shows that increasing clover contents of pasture is likely to lead to increases in profitability of farms, but doing so in practice is difficult.

Perennial ryegrass pastures are hungry for extra nitrogen in spring (Fasi *et al.,* 2008). This is because nitrogen often becomes the most limiting factor for plant growth due to replenished soil moisture levels. As temperatures warm and soil moisture begins to drive plant growth, more nitrogen is required to maintain a plant nitrogen content of around 3% (Peri *et al.,* 2002). Fasi *et al.,* (2008) showed the effects of increasing soil nitrogen levels on the growth of perennial ryegrass pastures in spring (Table 2.3). Pastures with 150 kg N/ha applied grew 8.3-10.3 kg DM/ha/°Cd compared with 2.5-4.2 kg DM/ha/°Cd ($P < 0.05$). Dry matter accumulation in response to added nitrogen ranged from 13.4 kg DM/kg N ('Aries', sown at 8 kg/ha) to 27.9 kg DM/kg N ('Revolution'). These nitrogen responses were similar to the 24 kg DM/kg N shown by Gillingham *et al.,* (1998) and 24.8 kg DM/kg N by Ball *et al.,* (1976).

Table 2.2: DM response (kg DM/kg N) and growth rate (kg DM/ha/°Cd) of 'Revolution', 'Cannon LE' and 'Aries' (sown at 8, 12 and 15 kg/ha) perennial ryegrass (Fasi *et al.,* 2008).

Perennial ryegrass cv.	DM response (kg DM/kg N)	Growth rate (kg DM/ha/°Cd)	
		0 kg N/ha	150 kg N/ha
'Aries' @ 8 kg/ha	13.4 bcd	4.2	8.3
'Aries' @ 12 kg/ha	20.1 ab	3.1	9.1
'Aries' @ 15 kg/ha	19.4 abc	2.7	8.7
'Cannon LE'	19.0 abc	3.7	9.4
'Revolution'	27.9 a	2.5	10.3

Increasing legumes in pasture can also reduce fertilizer costs on farms by fixing atmospheric nitrogen (Lucas *et al.,* 2010). Legumes form a symbiotic relationship with *Rhizobium* bacteria. These bacteria allow atmospheric nitrogen fixation through root nodules in legumes. Fixed nitrogen is then released into the soil, where it becomes available for other non-legume pasture species to use, which results in increased growth. Lucas *et al.,* (2010) estimated the amount of N in subterranean and white clovers that was derived from the atmosphere (fixed nitrogen) in an experiment at Lincoln University. They showed both species of clover to fix 28 kg N/ t clover DM (+/- 0.7 kg N). Subterranean clover fixed 81 kg N/ha/year compared with 46 kg N/ha/year for white clover. Assuming a Urea cost of $476/ t (Ravensdown, 2017), a tonne of legume herbage fixing 28 kg N/ha would be fixing $29 worth of N/ t DM. For an average dairy farm of 144 ha (Dairy NZ, 2014), growing ryegrass/ clover pastures producing 16.4 t DM/ha with a 25% clover content (Harris *et al.,* 1997), this would equate to $17,106 worth of fixed nitrogen.

2.2.4 Limitations of white clover in dairy pastures.

2.2.4.1 Growing season

The early spring legume content of dairy pastures is limited by the growth season of white clover. Dairy farms require high quality, high yielding pastures from the beginning of lactation. On most dairy farms, lactation begins in early August (Dairy NZ, 2014). White clover growth rates decrease due to cooling temperatures from May, with the lowest growth rates in August (Smetham, 1972). Growth rates then begin to increase with warming temperatures in September and October. Smetham (1972) showed the growth rate of two white clover cultivars from April through to October at a range of experimental sites. White clover produced 15.1 g DM/plant in May, before growth rates decreased to produce 8.63 g DM/plant in August. Growth rates then increased, producing 13.3 and 26.0 g DM/plant in September and October, respectively. The slow growth rates in August are a limitation of white clover in dairy pastures. Lactating cows need high quality feed from the beginning of lactation to maximize milk production. However, white clover cannot fill this need until growth rates increase in September/October. This creates a need for a high quality, winter active pasture species to be added to dairy pastures.

2.2.4.2 Clover root weevil (Sitona lepidus).

Another issue effecting white clover content in dairy pastures is insect pressure, in particular from clover root weevil (CRW). CRW is a palaearctic species that was first detected in the upper North Island in 1996, gradually moving south until it reached the bottom of the South Island in 2010 (Hardwick et al., 2016). Adult weevils feed on the leaves of clover, and can reduce the establishment of seedlings. However, the root-feeding larvae cause the most damage by reducing nitrogen fixation and root growth, weakening or killing the plant. CRW damage is estimated to cost NZ$ 2.4 billion p.a. CRW is biologically controlled to economically sustainable levels with a parasitic wasp that lays eggs inside adult CRW. Once hatched, the larvae eat the insides of the CRW. Further, once parasitized, adult CRW instantly become sterile, meaning they cannot produce more larvae. Hardwick et al., (2016), estimated the parasitoid to be worth $2.3 million p.a to Southland dairy farms.

Subterranean clover was shown to be more resistant to CRW when compared with white clover (Crush *et al.,* 2007). This was due to high concentration of isoflavnoids in the roots and shoots of subterranean clover. Isoflavonoids are thought to act as a deterrent to CRW feeding. Crush *et al.,* (2007) showed the effects of CRW presence on 'Denmark' and 'Leura' subterranean clover, striated clover (*Trifolium striatum*), cluster clover (*T. glomeratum*), suckling clover (*T. dubium*) and white clover in a glasshouse experiment. 'Denmark' subterranean clover root and shoot dry matter were reduced by 7.5% and 11.5%, respectively (Table 2.7). This compared with reductions in white clover root and shoot dry matter of 48.4% and 59.9%. Nitrogen fixation was also higher ($P < 0.01$) in subterranean clover than all other clover species when CRW was present. 'Denmark' subterranean clover fixed 99.2 mg N/plant, compared with 63.6 mg N/plant for white clover (LSD = 13.1). Isoflanovoid concentrations were higher ($P < 0.001$) in subterranean clover than other clover species. Root biochaninA levels in 'Denmark' and 'Leura' subterranean clover were 4.47 and 5.55 mg/g DM, respectively. In comparison, root biochaninA concentrations were 0.30, 0.11 and 0.14 mg/g DM for striated, clustered and suckling clover, respectively (LSD = 0.76). These results show the greater resistance of subterranean clover compared with white clover, which points to the potential for its use as a pasture legume in areas where CRW causes significant damage on white clover populations.

Table 2.3: Root BiochaninA concentration (mg/g DM), N fixation (mg N/plant) and reductions in shoot and root dry matter (%) of clovers subjected to CRW (adapted from Crush *et al.,* 2007).

Clover	Reduction in shoot DM	Reduction in root DM	Root biochaninA concentration (mg/g DM)	N fixation (mg N/plant)
'Denmark' sub clover	11.5%	7.5%	4.47	99.2
'Leura' sub clover	13.8%	9.8%	5.55	26.1
Suckling clover	40.4%	38.6%	0.30	53.0
Striated clover	51.2%	35.8%	0.14	20.2
Clustered clover	56.4%	44.1%	0.11	13.3
White clover	59.9%	48.4%	Not tested	63.6
LSD (5%)	-	-	0.76	13.1

2.3 Subterranean clover.

Because of the limitations of white clover, caused by its susceptibility to clover root weevil and limited growth in early spring, there is a need for another legume species to be added to dairy pastures. Subterranean clover is a winter annual legume that germinates in autumn, then produces high quality legume herbage in early spring before flowering and setting a seed in late spring, after which the plant dies. This allows the plant to avoid the summer dry period, making it a useful legume in dryland farming systems. If established, subterranean clover could provide high value feed for dairy cows in early spring, bridging the gap between the start of lactation and when white clover growth increases in October.

No literature has been published on the use of subterranean clover as a legume in dairy pastures. All data on subterranean clover in this review comes from dryland farming systems. This means data must be interpreted in a dairy farming context to be able to understand the potential of subterranean clover for dairy systems. The annual nature of subterranean clover may not suit a dairy system, which is what this research is designed to investigate.

2.3.1 History.

Subterranean clover was first recognised in New Zealand in the early 1900s, when it was found growing near Auckland (Smetham, 2003). The species was regarded as a weed until the mid-1920s, after which it became a popular annual legume used in easier hill country. However, the popularity of lucerne that began in the 1960s meant little research was carried out on the plant in New Zealand until the late 1990s. More extensive recent research has been carried out, including the use of subterranean clover in the nine year 'Max-Clover' experiment at Lincoln University (Brown *et al.,* 2006). Due to the favourable New Zealand climate and large variability between subterranean clover cultivars, there are still many areas that require further research (Scott, 1971). These include further evaluations on current cultivars as well as areas of potential use, such as it being an additional legume in dairy pastures.

2.3.2 Cultivar choice

Subterranean clover has three main sub species; *brachycalycinum, subterraneum* and *yanninicum* (Wright, 2015). Sub species *yanninicum,* and *subterraneum* are typically found in slightly acidic soils (pH 5.5 – 6.5), whereas *brachycalycinum* is typically found in neutral soils (pH 6.5 – 7.5). *Yanninicum* was also reported to have a greater tolerance to waterlogging than *brachycalycinum* or *subterraneum.* From use in a dairy farming system, any of the three sub species would be suitable, providing soils are light (e.g. silt loams). However, where there are deep, heavy soils with high rainfall, the *yanninicum* sub species could provide greater tolerance to waterlogging.

In a dryland system, subterranean clover cultivar choice should not be based solely on dry matter production due to the need for the plant to set sufficient amounts of seed in spring to allow it to persist the following season (Scott, 1971). Subterranean clover needs to flower and set seed before the plant dies off once moisture stress occurs. Moisture stress will begin to occur at different times in different areas, meaning that a late flowering cultivar that has sufficient moisture to flower and set seed in Napier (annual rainfall 800 mm) might struggle to do so in North Canterbury (650 mm). Scott (1971) evaluated dry matter and seed yields of six different cultivars sown in Wakari (annual rainfall <800 mm). He showed that the earlier flowering cultivars such as 'Geraldton' (1638 kg DM/ha) grew less spring dry matter than late flowering cultivars such as 'Tallarook' (3572 kg DM/ha). However, 'Geraldton' set more seeds/m² ($P < 0.05$) than all other cultivars (Table 2.4). In a dairy system, seed set is a secondary priority compared with herbage production. This means cultivar choice can be based on herbage production alone.

Table 2.4: Seed production and dry matter yield of early and late flowering subterranean clover cultivars. Adapted from Scott (1971).

Cultivar (earliest to latest flowering)	Seeds/m²	Spring dry matter yield (kg DM/ha)
Geraldton	1,774 a	1639 c
Yarloop	273 d	3576 b
Wogenellup	863 b	5777 a
Clare	749 bc	6655 a
Mt Barker	573 c	4135 b
Tallarook	272 d	3752 b
S.E Mean	+/- 98	356

2.3.3 Herbage production

Later flowering subterranean clover cultivars produce greater herbage yields than early flowering cultivars, given adequate moisture and a successful seedling establishment the previous autumn (Widdup & Pennel, 2000). This is because late flowering cultivars produce more leaf-bearing nodes before flowering takes priority of assimilates. Widdup

& Pennel (2000) showed the herbage yields of 20 subterranean clover cultivars, ranging from early to late flowering. They showed a strong relationship (r^2= 0.70) between flowering time and herbage yields in the first growth season. Spring herbage yields ranged from 2,500 kg DM/ha for early flowering cultivars to >7000 kg DM/ha. However in following seasons, herbage yields became more dependent on factors such as seed set, hard seed levels and seedling establishment. This highlights the importance of appropriate cultivar selection when aiming to maximise the persistence of subterranean clover. No connection was made in this paper between the time of flowering and the timing of growth. However, Richardon (2003) showed eight subterranean clover cultivars to have similar base temperatures, which indicates that growth is likely to begin when temperature allows, as opposed to a relationship between flowering time and growth. These findings are important in a dairy system, as farmers would be looking to bridge a feed gap from early August to mid-October. If the timing of growth was dependent on flowering time (e.g. early flowering = early growth, late flowering = late growth), then farmers would need to choose cultivars that have a growth pattern that fits into the feed gap. The findings by Richardon (2003) mean that farmers can choose cultivars based solely on herbage production, as the timing of growth will depend on temperature. If seed was to be sown every year, farmers also would not need to factor seed set into their choice of cultivar. Large leaved, mid-late flowering cultivars such as 'Woogenellup' and 'Antas' (Lucas & Moot, 2016) would most likely be best suited to maximise herbage production on dairy farms.

Subterranean clover also showed higher cool season growth compared with lucerne (*Medicago sativa L.*.) and a resident pasture (Smetham & Jack, 1995). Subterranean clover has a low base temperature, meaning it will keep accumulating thermal time through winter, especially in areas with mild winters. To calculate thermal time requirements for different stages of crop growth, Richardon (2003) used 0 °C as the base temperature for subterranean clover. Smetham & Jack (1995) showed the dry matter accumulation of 18 different subterranean clover cultivars in a trial at Lincoln University. These cultivars were compared with lucerne and a resident pasture made up of striated clover clustered clover and hairgrass (*Vulpia spp*). Six of the pure subterranean clover swards accumulated 4103 – 5231 kg DM/ha from autumn to spring. This compared with

3310 kg DM/ha for lucerne and 764 kg DM/ha for the resident pasture over the same period. Smetham & Jack (1995) noted that the low lucerne yield was due to lower than normal rainfall over autumn and winter. This highlights the ability of subterranean clover to utilise soil moisture in cooler months before it runs out in early spring in drought situations. In a dairy system, moisture deficit in early spring is not a common issue, as most dairy farms either have reliable irrigation in spring or high rainfall.

Cocksfoot with subterranean clover pastures have been shown to have greater dryland production than other grass based pastures (Mills *et al,* 2008). This is due to the ability of both species to persist in drought or low moisture conditions. Further, the cool season growth of subterranean clover complements the warm season growth of cocksfoot. This cool season growth is what makes subterranean clover appealing for use in dairy systems. In contrast, the summer dormancy of subterranean clover prevented the large leaved cocksfoot from competing with the clover for light interception. In a dairy system, this summer period is when white or red clover (*Trifloium pratense*) would be expected to be in full production.

Herbage production was shown to increase with earlier sowing. This is due to a greater thermal time accumulation in earlier sown swards resulting in greater growth. Moot *et al.,* (2003) showed subterranean clover that germinated in March yielded 7000 kg DM/ha by mid-September, compared with 1800 kg DM/ha for plants that germinated in May. Although Moot *et al.,* (2003) did not show any data on difference in seed set between germination dates, higher yielding swards likely had more reproductive sights, which would likely have resulted in greater seed set. The information for seed set isn't fully relevant for subterranean clover in a dairy system, as seed could be re-sown each year. However, results showing greater herbage yields from earlier sowing are of importance, as maximising yields would be the main aim when adding subterranean clover into a dairy pasture. Most dairy farms would also not have to worry about false strike (germination from drought season rain), as irrigation removes any moisture deficit from spring through to autumn.

The temporal pattern of yield and botanical composition of different clover was summarized for six dryland pastures over five years in an experiment at Lincoln University (Mills *et al.,* 2008). The pastures included cocksfoot (cf)/subterranean clover (sub), cf/white clover (wc), cf/balansa clover (*Trifolim michelianum*) (bal), cf/Caucasian clover (*Trifloium ambiguum*) (cc), perennial ryegrass (rg)/wc and pure lucerne. Cf/sub pastures were shown to be the highest yielding grass based pasture in terms of dry matter production over the course of the experiment (Figure 2.1). This was due to subterranean clover producing herbage in the cool season when moisture levels were high. In a dairy system, sub clover could still increase early spring yields, while the summer production of ryegrass/white clover pastures would not be moisture limited as they were in this experiment. Cf/sub pasture yields were greater ($P < 0.05$) or similar to all other grass based pastures over each of the five growing seasons. Cf/sub pastures grew 8.3 kg DM/ha/°Cd in spring, which was greater ($P < 0.05$) than the 6.5 kg DM/ha/°Cd for all other grassed based pastures. These results show that subterranean clover pastures were the highest yielding dryland grass-based pasture. If successfully established, the cool season growth of subterranean clover could suit a dairy system well, without causing changes in grazing management in spring.

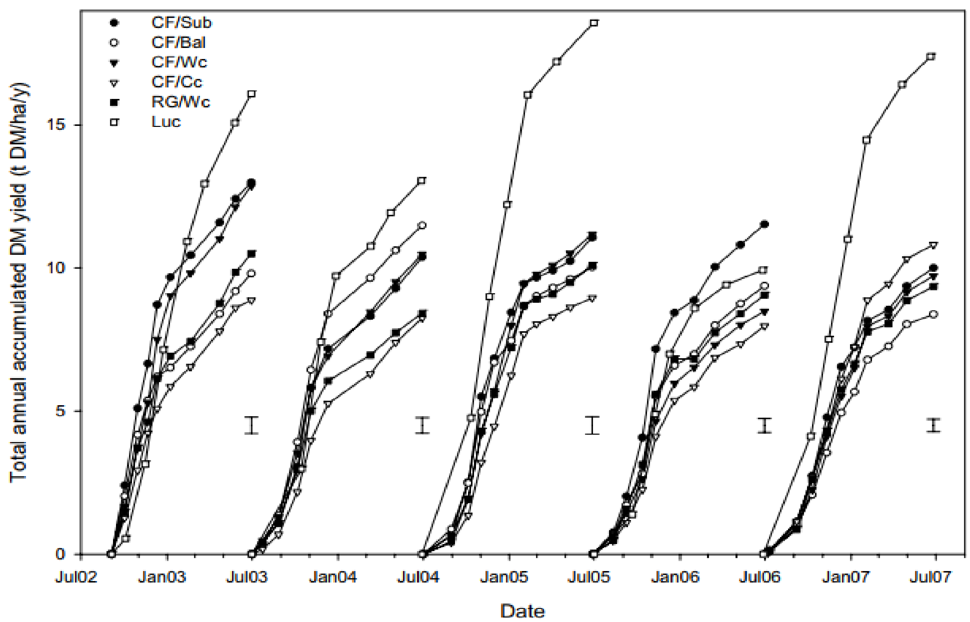

Figure 2.3.1:Total accumulated annual dry matter (DM) production of CF/Sub, CF/Bal, CF/Wc, CF/Cc, RG/Wc and lucerne pastures for five growth seasons (Mills *et al.,* 2008).

2.3.4 Seed production and seed set.

Overstocking subterranean clover in late spring can reduce seed set (Ates *et al.* 2006). This is because subterranean clover requires periods of low grazing pressure during seed set in late spring to allow burrs to form. Under heavy grazing, tips of runners can be grazed off, leaving fewer sites for burrs to form. Ates *et al.* (2006) compared the effects of sheep stocking rate on subterranean clover persistence in a tall fescue pasture mix at the Ashley Dene dryland research farm. Ewes were stocked at 20 and 10 ewes per ha with twin lambs. Results showed 62% fewer burrs per m^2 under high stocking rates compared with low stocking rates. This experiment was carried out over a spring in which drier than normal conditions were experienced meaning the subterranean clover plants wilted about four weeks earlier than normal. This would have had a negative effect on runner length and the number of burrs produced. Therefore it is possible that the subterranean clover would have coped under higher stocking rates had it not also been under moisture stress. The conclusions drawn by Ates *et al.* (2006) were backed up by Smetham & Dear (2003). They measured the effects of hard, lenient and no grazing on seed yield of three pure subterranean clover swards (Table 2.6). Hard grazing produced 71 kg of seed per ha, 94% lower than the 1,254 kg produced by the sward with no grazing. Lenient and hard grazing both had post grazing herbage residuals of 1,613 and 1,405 kg DM/ha, however lenient grazing produced 253 kg/ha more seed than hard grazing. These results show the negative effects of grazing too hard during seed set. However, cattle graze higher from the ground than sheep, meaning they are less likely to graze burr sites. This suggests dairy grazing could allow some seed set, although this would not be the main aim for subterranean clover in dairy pastures. Instead, it may be profitable to re-sow seed every year if sufficient herbage was produced to increase milk production and nitrogen fixation. This means any seed that is set and regenerates the following year would be seen as a bonus.

Table 2.5: Effect of three levels of grazing on herbage mass in spring, and seed yield the following autumn, of subterranean clover (Smetham & Dear, 2003).

Grazing intensity	Seed yield (kg/ha)	Herbage mass (kg/ha)
Nil	1,254	5,587
Lenient	324	1,613
Hard	71	1,405

2.3.5 Subterranean clover establishment.

For subterranean clover to increase spring legume content of pastures, successful establishment is critical. In dryland systems, the timing of moisture (autumn rainfall) and seed set the previous season are two important factors determining seedling regeneration. However, in a dairy system, false strike (germination as a result of drought season rainfall) wouldn't be a problem due to the use of irrigation. Seed set would also be unlikely to be an issue, as it could be profitable for seed to be sown each year. In a dairy system, the main factors influencing successful establishment would most likely be germination percentage and competition of established pasture species after emergence. When over-drilling is the method of sowing, as is the case in this research (Section 3.1.4), rapid establishment is critical to prevent seedlings from being out-competed for resources by established pasture species.

2.3.5.1 Germination.

The germination percentage of subterranean clover is influenced by temperature and moisture (Hampton *et al.,* 1987). This is because germination starts by the seed imbibing water, which occurs after the break down of the seed coat. This activates enzymes within the seed. Temperature affects the rate of activity in these enzymes, therefore affecting the speed of the germination process. Hampton *et al.,* (1987) showed that subterranean clover germination was inhibited when soil moisture levels were below 10-15%. Murray (2012) noted that subterranean clover didn't germinate in soil temperatures above 20 °C. Moot *et al.,* (2000) showed that subterranean clover required 45 °Cd to germinate and 120 °Cd to emerge (assuming a base temperature of 0 °C). In a dairy system, the information from Hampton *et al.,* (1987) would be irrelevant, as moisture is not often limited due to irrigation. However, the information by Murray (2012) and Moot *et al.,* (2000) can be used to determine a sowing date. Ideally, sowing should occur once soil temperatures are below 20 °C.

2.3.5.2 Soil fertility.

Soil fertility is important for establishing seedlings. Nutrient deficiencies can cause restrictions to root growth. This can result in establishing plants being out-competed for resources, leading to poor establishment.

Increasing phosphorus levels in the soil has been shown to increase legume production (Gillingham *et al.,* 1998). Legumes are generally more responsive to phosphorus applications than non-legume species. This is because plants will only be limited by their most limiting factor. Non-legume species are often limited by soil moisture or nitrogen availability due to fibrous roots and their inability to fix atmospheric nitrogen. However, legumes such as subterranean clover that have tap-roots and are self-sufficient for nitrogen due to fixation are more likely to be limited by phosphorus. This means when phosphorus is applied, legumes can respond quickly. In contrast, non-legume species that are limited by nitrogen won't respond to phosphorus applications until increased nitrogen fixation removes the nitrogen limitation. Gillingham *et al.,* (1998) showed the effects of applying phosphorus on the production and botanical composition of grass/clover pastures on hill country in southern Hawke's Bay. Pastures with low P application produced 316 kg clover DM/ha, compared with the 1111 kg clover DM/ha in the high P treatment ($P < 0.001$). Clover content in the low P pastures was 17%, compared with 31% in the high P treatments. In dairy pastures, soil fertility is typically high. Plant available phosphorus (Olsen P) is normally around 30-40 (Rowarth *et al.,* 1996), meaning clover is unlikely to be limited by phosphorus. Limitations to subterranean clover growth would more likely be due to competition from other pasture species.

Acidic soils were shown to have a negative effect on the herbage production and nitrogen fixation of subterranean clover (Hayes *et al.,* 2008). Acidic soils (pH < 5.2) have increased concentrations of exchangeable aluminium (Al). High concentrations of exchangeable Al can cause Al toxicity in plants. This can lead to symptoms such as horizontal root growth, decreased root dry matter, decreased nodulation in legumes as well as a general decrease in plant productivity. Hayes *et al.,* (2008) showed the effects of lime application on the growth and nitrogen fixation of subterranean, balansa and gland clover. This experiment was carried out in NSW, Australia. Liming increased soil pH, resulting in herbage production of all clovers increasing by 18-22%. Liming also increased the amount of herbage N derived from nitrogen fixation by 29-40% in subterranean clover. These results were primarily due to increased root growth and nodulation, which was a result of lower exchangeable Al levels in the soil. Due to the high fertility and the easier application of lime in comparison to hill country soils, soil acidity isn't a problem on dairy soils.

2.3.5.3 Waterlogging.

Waterlogging of soil was shown to decrease the root and shoot growth of subterranean clover. This is because waterlogging fills soil pores with water, reducing the oxygen levels in the soil. This leads to a decrease in root respiration, therefore decreasing root density and depth. Francis and Devitt (1969) showed the effects of 21 days of waterlogging on six week old subterranean clover seedlings. They showed herbage growth to be reduced by 26%, while root growth was decreased by 44%. While waterlogging is rare in dryland pastures in New Zealand, the potential for waterlogging in dairy pastures is comparatively greater. This is due to irrigation maintaining soil moisture levels, meaning that excessive rainfall in autumn could result in waterlogging of soil. Waterlogging during autumn would coincide with when subterranean clover is establishing in a dairy pasture, and could reduce the establishment of subterranean clover within the pasture.

2.4 Methods for increasing clover content in spring.

2.4.1 Over-drilling as a method for increasing yield.

Over-drilling subterranean clover into established dryland pastures increased spring yields and legume content (Ates *et al.,* 2010). This is because the cool season growth of subterranean clover increased pasture growth rates in late winter and early spring. During this period, other pasture species have little growth. This means, once established, there was little competition for subterranean clover to grow. Ates *et al.,* (2010) over-drilled subterranean clover into permanent perennial ryegrass or cocksfoot based pastures at Ashley Dene. Data around pasture production and the variables that accounted for growth (moisture and temperature) were presented. In September 2007, pastures over-drilled with subterranean clover grew 37 kg DM/ha/day more ($P < 0.01$) than those without subterranean clover. Ryegrass-sub pastures grew 30% more ($P < 0.001$) than cocksfoot-sub pastures. When moisture was non-limiting in early spring, ryegrass/sub pastures grew 8.4 kg DM/ha/°Cd compared with 6.1 kg DM/ha/°Cd for ryegrass pastures without subterranean clover. These results show the effectiveness of over-drilling subterranean clover into established pastures. However there was no discussion of the potential for a similar method to be used in irrigated/high rainfall areas.

2.4.1.1 Issues with over-drilling subterranean clover.

Over-drilling straight into established pasture means that there is no physical control (e.g cultivation) of pasture pests such as slugs (*Deroceras reticulatum*) and grass grub (*Costelytra zealandica*). Slugs thrive in moist environments such as those found in a typical dairy pasture. This means they could be a problem during the establishment phase of over-drilled subterranean clover.

Slugs were shown to prefer to feed on white clover seedlings compared with ryegrass seedlings (Barker, 1991). This has been linked to the height and horizontal aspect of clover leaves compared with ryegrass. Barker (1991) showed the effects of slug numbers and sowing rate on the preference of slugs grazing newly established ryegrass/white clover pastures. Clover seedling weights decreased ($P < 0.05$) in treatments where slugs were present over the entire eight week trial period. In comparison, slugs had no effect on the weight of ryegrass seedlings after the first four weeks of the trial. Barker (1991) linked this to the slower establishment and vertical growth of white clover in the first eight weeks of establishment in comparison with ryegrass. They also linked these results to slugs preferring the horizontal aspect of clover leaves as opposed to vertical ryegrass tillers. This information shows that if slugs are present in a pasture, they are more likely to graze on clover than on ryegrass. Further, when seeds are over-drilled into existing pastures, they will likely be preferred by slugs than other pasture species as they will be lower in the sward than established pasture species.

2.4.2 Gibberellic acid.

Gibberellic acid (GA) is a plant hormone that activates dormant enzyme systems. Application of GA can stimulate out of season growth, or increase growth through the mobilisation of plant reserves. GA is also known to promote leaf and stem elongation, as well as promoting flowering (Matthew *et al.,* 2009). This occurs by GA triggering the degradation of DELLA repressor proteins. DELLA repressors restrain plant growth (Schwechheimer, 2008). Degradation of DELLA repressors relieves plant restraints, which increases growth. Excessive rates of GA application (25-700 g/ha) have been shown to be uneconomic and also depressed plant growth after the initial increase from application (Matthew *et al.,* 2009). However, application rates of 5-10 g/ha have proven to be more economic and have shown less negative side effects (van Rossum, 2013). GA application could potentially be used to stimulate plant growth during early lactation on dairy farms.

GA increased spring dry matter accumulation and clover content (van Rossum, 2013). This is because GA promotes cell expansion, resulting in greater leaf area and stem development. This can lead to quicker canopy development in the sward, meaning greater light interception. van Rossum (2013) studied the effects of GA application on botanical composition, yield and nutritive value of perennial ryegrass/ white clover pastures in autumn and spring. Pasture height was 40% taller in pastures treated with 20 g ProGibb SG/ha in spring compared with pastures left untreated. GA application increased white clover yields by 53% compared with controls. Pasture yields in treated pastures were 1009 kg DM/ha, compared with 666 kg DM/ha in untreated pasture, 28 days after GA application in early August 2012.

Gibberellic acid caused a change in the chemical composition of treated plants (Blacklow & McGuire, 1971). This was because the plants failed to synthesise compounds (e.g. chlorophyll and crude protein) at a sufficient rate to maintain concentrations within the plant. Blacklow & McGuire (1971) found a decrease in chlorophyll concentration of 30-35% in tall fescue when GA was applied, resulting in chlorosis. Brown *et al.,* (1963) said that a reduction in crude protein content was the most consistent effect GA had on chemical composition (no data given). They also concluded that the reduction in crude protein was usually offset by an increase in yields, resulting in an increase in crude protein per hectare. However, reduced crude protein levels have been shown not to have an effect on milk yield. Reid *et al.,* (2015) showed the effect of varying dietary crude protein levels on milk solid yield. Cows were fed on high CP (302 g CP/kg DM), medium CP (202 g CP/kg DM) or low CP (101 g CP/kg DM) diets. There was no difference ($P > 0.05$) on milk solid yield between the different diets. Further, Bryant *et al.,* (2016) showed combining nitrogen fertilizer with GA application to ryegrass pastures nullified the negative effects on pasture quality that are associated with GA. This indicates that the nitrogen fixation from legumes could allow legumes to maintain pasture quality with GA application. This means that GA application could increase clover DM yields without reducing quality, which would result in greater milk solid production.

2.5 Conclusions.

- Increasing energy intake of dairy pastures can increase milk solid production of dairy cows.
- Increasing clover content can increase the nutritive value of dairy pastures, therefore increasing milk solid production.
- Increasing clover content will increase nitrogen fixation by about 28 kg N/ t legume DM.
- White clover is limited in dairy pastures due to low early spring growth and susceptibility to clover root weevil attack.
- The high early spring growth of subterranean clover makes it a potential pasture species to bridge the gap in legume growth from the beginning of lactation until white clover grows in October.
- No literature on the use of subterranean clover in irrigated pastures has been published. However, many of the limitations of subterranean clover that have been identified in dryland environments such as persistence due to seed set, moisture stress in late spring and soil fertility would not apply in dairy pastures.
- Over-drilling subterranean clover would be a simple way of adding it to a dairy pasture. However, this could leave establishing seedlings susceptible to slug damage.
- Gibberellic acid could potentially be used to promote clover dry matter in early spring.

3 MATERIALS AND METHODS

Three experiments were used in this research. Experiment 1 and Experiment 2 both investigated the potential for over-drilling subterranean clover into established ryegrass pastures as a method for increasing early spring dry matter yields and legume content. The null hypothesis for both of these experiments was that early spring dry matter yields and legume content would not be increased by over-drilling subterranean clover in autumn. The third experiment investigated the use of gibberellic acid (GA) as a method for increasing dry matter yields of pure subterranean clover. The null hypothesis for Experiment 3 was that GA application would not increase subterranean clover dry matter yields

3.1 Experiment 1 – Iversen field (I3).

3.1.1 Experimental location.

Experiment 1 was in Iversen Field, paddock I3 at Lincoln University. The experimental area was 37 by 20 m in the southeast corner of the paddock. The paddock contained an established perennial ryegrass pasture, sown as pure ryegrass in spring 2015. The soil is a Wakanui silt loam (Landcare Research, 2017). Olsen Phosphorus (Olsen P) level was 13 mg/L, and pH was 5.7 (Table 3.1). Long term average rainfall at the site is 630 mm (Section 3.4). There were patches of volunteer white clover throughout the paddock, as well as small amounts of Caucasian clover. The Caucasian clover was from when the paddock was sown in pure Caucasian clover in 2013. The clover content in the trial area was less than 5% at the beginning of the experiment.

3.1.2 Experimental Design.

Experiment 1 at Iversen field used a randomised complete block design. Three different cultivars of subterranean clover (‘Woogenellup’, ‘Antas’ and ‘Denmark’) were over-drilled into the established ryegrass pastures, as well as a control treatment where no seed was sown, although the drill was still run through the pasture. This gave four treatments, with four replicates per treatment giving a total of 16 plots. Each plot was 2.1 by 20 m (Appendix 1).

3.1.3 Thousand seed weight.

Thousand seed weights were measured for each cultivar on 15th March 2017, and are summarized in Table 3.1.

Table 3.1: Thousand seed weight (g) and seeds/m² of three subterranean cultivars used at Experiment 1 and Experiment 2.

Cultivar	Thousand seed weight (TSW) (g)	Seeds/m² at 10kg/ha
Antas	9.40	106
Woogenellup	10.2	98.0
Denmark	6.91	145

3.1.4 Sowing

Prior to sowing the pasture at Iversen Field was reasonably open with less than 5% legume content, although no botanical composition measurements were taken prior to sowing to quantify this. There was lots of dead plant matter at the base of the sward, which was likely left over plant material from a previous cut & carry or balage harvest. The pasture was mown to 1700 kg DM/ha, with all cut pasture taken away from the site before sowing. Each cultivar was over-drilled at a rate of 10 kg/ha on the 17th March 2017, using a 2.1 m wide Flexiseeder plot drill at a target depth of 1-2 cm. Drill rows were 15 cm apart. The weight of seed required for a sowing rate of 10 kg/ha was calculated to be 42 grams of uninoculated seed per plot. Seed for each plot was weighed and placed in separate containers. Before sowing each plot, the seed for that particular plot was fed into the drill. Initially we planned to heavy roll the experiment area after sowing.

However heavy rain immediately after sowing (Figure 3.1) meant the potential to cause damage to the experimental area was too high, so no rolling occurred.

3.1.5 Defoliation management

Grazing was simulated by cut and carrying pasture from the experimental area. The first cut was taken on 2nd May 2017 (48 DAS). The residual herbage mass was 1700 kg DM/ha. A second defoliation began on the 10th August 2017. About 40 ewes were put into I3. The pre-grazing herbage mass was 2400 kg DM/ha. Ewes were taken off the paddock on the 16th August. The post grazing herbage mass was again 1700kg DM/ha. All pasture measurements were taken using a rising plate meter. Thirty readings were taken with the plate meter, which then gave an average dry matter yield using the equation ("clicks" x 140 + 500) (M. Smith, pers. comm. 2nd May 2017).

3.1.6 Insect/slug control.

Experiment 1 was damaged by slugs and grass grub. Slug damage was observed shortly after seedlings emerged. The experimental area received a dose of Measurol slug and snail bait (ai 20 g methiocarb/kg) on the 13th April at 6 kg/ha. Measurol has an active duration of 14 days, and it was recommended to use a follow-up dose. Grass grub was then discovered at the end of April. Dew 600 was used to kill the grass grub, sprayed on the 8th May. This spraying was used as an alternative to the recommended follow up dose of Measurol. Dew 600 contains an active ingredient of 600 g/l of Diazinon. A combination of 4 L/ha sprayed with 200 L of water/ha was used on the experimental site. This application rate is higher than commonly used, but was recommended by David Jack (pers. comm.) with an expectation it would kill slugs. Grass grubs were still present at the end of May, so a second application of Dew 600 at 1 L/ha was applied on the 9th June. Slugs were found again in early August. Measurol was applied at 6 kg/ha on the 17th August across the whole trial paddock.

3.1.7 Establishment counts.

A seedling establishment count was carried out at Iversen field on 21st April 2017, 35 days after sowing (DAS). Originally, we had planned to carry out the establishment count 14 DAS. However, the slug damage meant that some emerged seedlings were unlikely to survive (Plate 3.1), so the establishment count was delayed until 35 DAS. In each plot (excluding control plots), a seedling was marked by placing a wooden peg in the ground beside it. From the marked seedling, 20 cm was measured along the drill row and seedlings in this drill row were counted and recorded. Seedlings were deemed to have established if they had produced a spade leaf or a trifoliate. This process was carried out four times in each plot, with each marker being spaced evenly down the plot. Seedling counts were multiplied by five to give mean seedlings/m of drill row for each plot, then multiplied by (1/0.15) to give seedlings/m².

Plate 3-1: Subterranean clover seedlings showing cotyledon damage by slugs on the 07/04/2017 at paddock I3, Ivesen field, Lincoln University, New Zealand.

3.1.8 Leaf appearance rate.

Leaf appearance rate of the subterranean clover plants was measured. Two plants were marked in each plot. Every 7-10 days, the number of fully emerged leaves on each plant was counted and recorded. A leaf was deemed to have fully emerged once each of the leaflets on the trifoliate had begun to unfold. Twelve leaf counts were taken between 5/5/2017 and 9/8/2017.

3.1.9 Pasture cuts.

Two 0.2 m^2 quadrat cuts were taken from each plot on the 11th October 2017. At least 50 g fresh weight of each sample was sorted into different botanical components of the pasture (grass, white clover, subterranean clover and weeds). Each botanical component was bagged, and placed in a tray along with the rest of the sample (bulk). The samples were then dried in an oven at 60 °C for at least 48 hours. Samples were then removed and weighed. Data on botanical composition and dry matter yields were recorded.

3.1.10 Soil tests

Soil tests were taken from Experiment 1 and Experiment 2, as well as paddock W5 at Experiment 2. A 75 mm soil corer was used to sample 500 – 1000 g of soil from across the paddock. These samples were stored in plastic bags and analysed by R. J. Hill Laboratories. Results are shown below in Table 3.2

Table 3.2: Soil test results for Experiment 1 (I3) and Experiment 2 (A6 & W5), and recommended soil test values (McLaren & Cameron, 1996).

Block	pH	Olsen P (mg/L)	K (me/100 g)	Ca (me/100 g)	Mg (me/100 g)	Na (me/100 g)
I3	5.7	13	0.52	7.1	1.14	0.15
A6	6.5	34	0.39	13.4	1.15	0.07
W5 (Bot)	6.8	79	1.56	16.6	2.02	0.07
W5 (Mid)	6.6	49	0.7	15.6	1.69	0.11
W5 (Top)	6.6	86	1.24	17	1.92	0.07
Recommended values	6.2	30	0.60	10.0	1.60	0.50

3.2 Experiment 2 - Invernia

3.2.1 Experimental location.

The second experiment was at Invernia, a 2,500 ha dairy and sheep farm in North Otago. Invernia is fully irrigated using border dykes. The paddock in which the experiment took place was Airport 6 (A6). This paddock was sown on 15/10/04 with 10 kg/ha of 'Sterling' perennial ryegrass and 3 kg/ha of a non-commercial Caucasian-white clover hybrid.

A second paddock, Woolshed 5 (W5), was used to run the drill out after the sowing of each cultivar was completed in A6. W5 was sown on 20/9/07 with 10 kg/ha 'Aberdart' perennial ryegrass, 8 kg/ha 'Endura' Caucasian clover, 2 kg/ha 'Tribute' white clover and 1 kg/ha 'Viking' timothy.

3.2.2 Experimental design.

Airport 6 used a randomised complete block design. Three different cultivars of subterranean clover ('Woogenellup', 'Antas' and 'Denmark') were over-drilled into established ryegrass pastures, as well as a control treatment where no seed was sown, although the drill was still run through the pasture. This gave four treatments, with four replicates per treatment giving a total of 16 plots. Each plot was 6 m (two drill widths) by approximately 100 m (Appendix 2).

Woolshed 5 had no experimental design. Roughly a third of the paddock was sown in each cultivar, with a 5 m gap between each cultivar.

3.2.3 Sowing.

Seed was over-drilled on the 5th March 2017 at 10 kg/ha, using a 3 m wide Taege 300BT121 direct drill. Drill rows were 121 mm apart. For each cultivar, ~10 kg of seed was put in the drill, and the drill was calibrated to sow the seed at 10 kg/ha. After calibration, all four replicates of each treatment (cultivar) were sown in Airport 6.

The drill was then run out in Woolshed 5. In Woolshed 5, the paddock was split into three similar sized blocks, and each cultivar was over-drilled into a single block until the drill was empty. After the drill was ran out, it was then cleaned with a seed vacuum before the seed for the next cultivar was put in. The sowing rate was calibrated/checked for each cultivar, and the same sowing process repeated until all three cultivars had been sown.

3.2.4 Grazing

At the Invernia trial, grazing was timed to fit in with the normal management of the dairy farm. The first grazing was on the 25th/26th March (20 DAS). 400 cows grazed Airport 6 between evening milking on the 25th and morning milking on the 26th (approximately 12 hours grazing). The first grazing was light, bringing the pasture down to 2200 kg DM/ha. The main aim of this grazing was to open up the pasture and allow light through to the seedlings. A second grazing of Airport 6 occurred on the 5th May 2017, 45 DAS. Ideally this grazing would have occurred earlier, but higher than usual rainfall throughout all of autumn (Figure 3.4.4) meant the paddock was very wet and the cows would likely cause pugging damage. The paddock was still wet/soft underfoot when the cows went in, which led to a lot of treading damage. The paddock was grazed down to 1800 kg DM/ha. A third grazing on the 3rd September brought the pasture down to 5-7 cm. No pre or post grazing dry matter measurements were able to be taken, although these residual estimates are typical of those used at Invernia.

Woolshed 5 was also lightly grazed down to 2200 kg DM/ha on the 26th/27th March. A second grazing on the 6th/7th May brought the paddock down to about 1800 kg DM/ha. Another grazing occurred on the 15th of August. The pasture was grazed down to an estimated 5-7 cm, however no pre or post grazing dry matter measurements were taken.

3.2.5 Establishment counts.

A seedling establishment count was carried out in Airport 6 at Invernia on 19th March 2017 (14 DAS). The same method used in Experiment 1 was used at Invernia. Seedlings were marked in a plan to take a second establishment count after the first grazing, to quantify the damage caused to the seedlings during grazing. However during the first grazing, the cows kicked every marker peg out of the ground, meaning it wasn't possible to take a second count beginning at the marked point. A post-grazing establishment count was attempted after the grazing on the 5th May. However the extent of the damage caused by the cows meant seedlings were unable to be found in many plots.

No establishment count was carried out in Woolshed 5.

3.2.6 Herbage accumulation measurements.

Post-grazing dry matter measurements for each plot were taken in Experiment 2 on the 5th May 2017, using a rising plate meter. The mean dry matter accumulation from 30 readings was taken for each plot. The same calculation described in Section 3.1.8 was used. The purpose of these measurements was to check if there was any difference in the dry matter accumulation among plots that could affect the results of the pasture cuts taken from the plots in spring.

The same method of pasture cuts used when harvesting Experiment 1 (Section 3.1.9) was used for Experiment 2. Pasture cuts were taken on the 3rd October 2017.

Pasture cuts from Woolshed 5 were taken on the 09/09/2017. The paddock was split into three blocks, running at right angles to the blocks of each cultivar. On visual inspection, there was subterranean clover growing well at the top and bottom of the paddock, but none was growing in the middle. The same method used when harvesting Experiment 1 (Section 3.1.9) was used for the observations from Woolshed 5.

3.3 Experiment 3 – Iversen Field

3.3.1 Experimental location.

The third experiment was in Iversen Field, paddock I2 at Lincoln University. The area consisted of 15 subterranean clover cultivars and one white clover cultivar ('Nomad') sown in a randomised complete block design on 16th April, 2015. The soil is a Wakanui silt loam (Landcare Research 2017) with available water holding capacity of ~150 mm/m. Olsen Phosphorus level was 13 mg/L and pH ($H2O$) was 5.4. Long-term annual average rainfall is 630 mm. Annual Penman potential evapotranspiration is 1094 mm and exceeds rainfall from September to April which results in a long-term potential soil moisture deficit of approximately 500 mm. However, higher than average rainfall (Section 3.4) meant that the area was not subjected to moisture stress for the duration of the experiment.

3.3.2 Sowing.

The area was cultivated prior to bare, non-inoculated seeds being broadcast by hand and raked in to plots of 4 m^2. Sowing rates were 20 g of seeds/m^2 for the sub clovers and 10 g of seeds/m^2 for 'Nomad'. Sufficient rain (44 mm) for germination occurred on 28th April. The commercial seeds were purchased from local suppliers and their characteristics are presented in Nichols *et al*., (2013).The non-commercial New Zealand line 'Whatawhata' (nucleus seed AK1332, Grasslanz, New Zealand) was selected based on previous evaluations by Widdup & Pennell (2000).

3.3.3 Experimental design.

Experiment 3 was originally sown in a randomised complete block design in 2015, with 15 different subterranean clover cultivars and one white clover cultivar. There were four replicates/blocks. Each block of the previous randomised complete block design was split in two, creating a cross-plot design. Six subterranean cultivars from the existing 15 were used in this experiment ('Leura', 'Antas', 'Denmark', 'Monti', 'Narrikup' and 'Woogenellup'). One side of each block was randomly allocated to receive 8 g/ha of gibberellic acid. This was applied to the plots on the 31st July 2017. Each plot was 2 m^2.

3.3.4 Height measurements.

The height of the pasture was measured for each plot on the 13th September 2017, using a 30 cm plastic ruler. The area measured was chosen based on it being the best representation of the height of the clover in the whole plot. This same method was used for a second measurement on the 10^{th} October 2017.

3.3.5 Pasture cuts.

The first pasture cut at Experiment 3 was taken on the 16^{th} August 2017. One 0.2 m^2 quadrat cut was taken, using hand shears, from each plot. The cut pasture was then placed in bags, before being sorted into subterranean clover, other clovers and weeds. Samples were dried for at least 48 hours in an oven at 60 °C. Once samples were dried, they were weighed and recorded. Further pasture cuts were taken on the 13^{th} September and 10^{th} October 2017. The same method was used from cutting through to weighing.

3.4 Weather data

Mean monthly weather data for 2017 was collected for Experiment 1 and Experiment 3 using the Lincoln University weather station. This station takes hourly readings of air temperature, surface temperature and rainfall. Long term average rainfall and air temperature data were collected from the NIWA National Climate database using the Broadfields weather station. Hourly thermal time calculations for Experiment 1 were made using the following calculation:

$$((Tmax + Tmin)/2) - Tbase$$

'Tmax' is the maximum hourly temperature, and 'Tmin' is the minimum hourly temperature. 'Tbase' is the minimum temperature at which plant growth occurs. For this calculation, a base temperature of zero was used (Moot *et al.,* 2000). The sum of hourly thermal time accumulation for each day was divided by 24, to give the daily thermal time accumulation.

Rainfall data for Experiment 2 were collected on-farm by the owner (Russell Hurst) throughout 2017. Mean monthly air temperature and long term average rainfall and air temperature were collected from the NIWA National Climate data base using the Oamaru

Airport weather station. Long term means were calculated using weather data from 1981-2010.

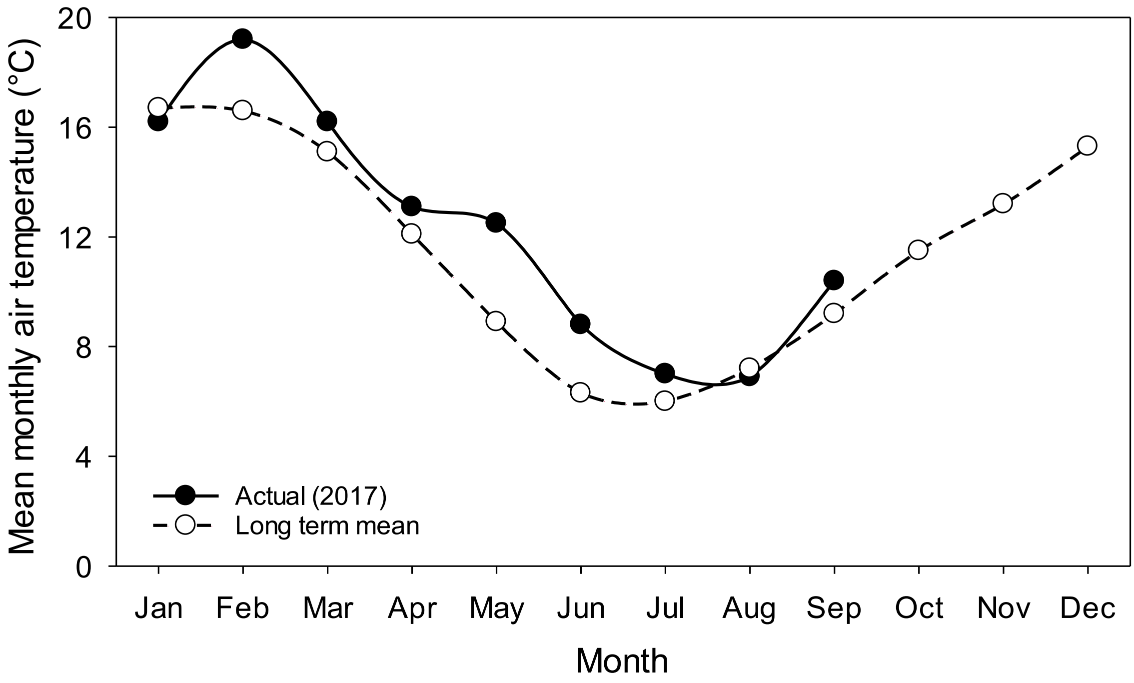

Figure 3.4.1: Long term (○) and actual (●) mean monthly air temperature (°C) at Lincoln

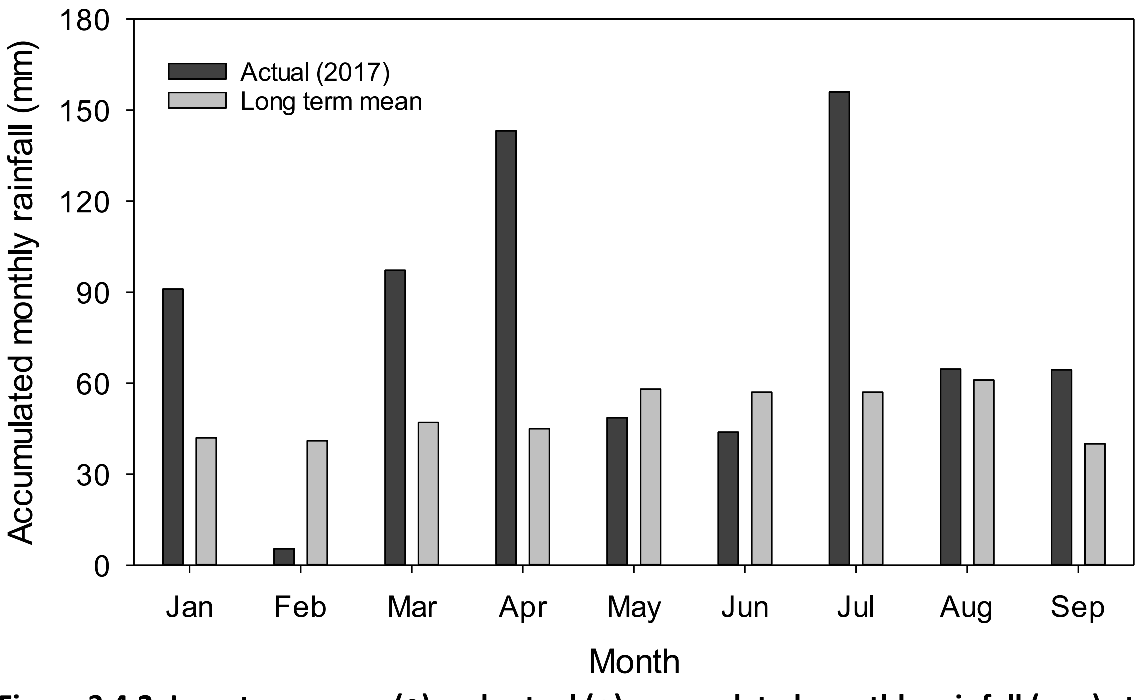

Figure 3.4.2: Long term mean (○) and actual (●) accumulated monthly rainfall (mm) at Lincoln University, Canterbury.

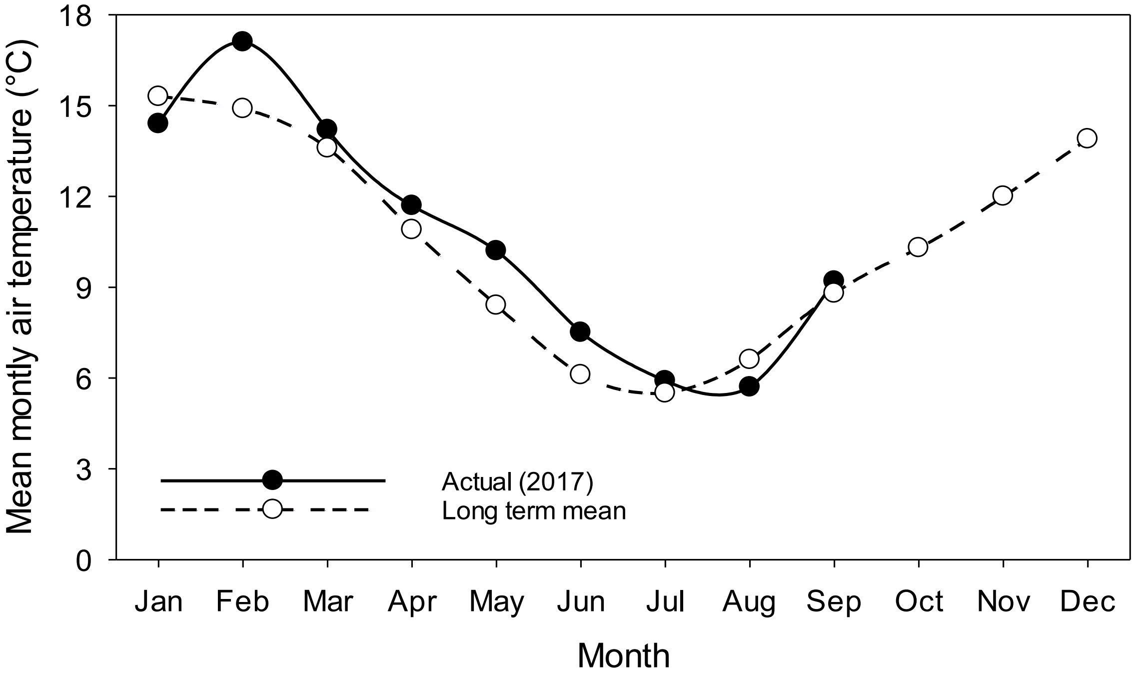

Figure 3.4.3: Long term (○) and actual (●) mean monthly air temperature (°C) at Invernia, North Otago, New Zealand.

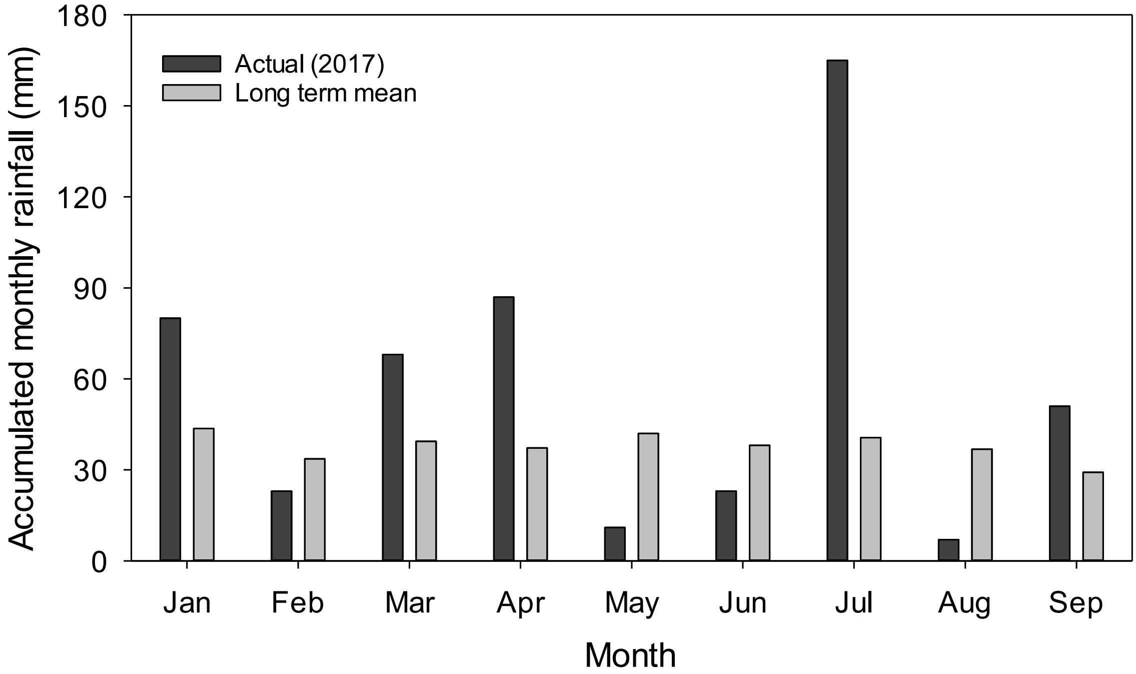

Figure 3.4.4: Long term mean (○) and actual (●) accumulated monthly rainfall (mm) at Invernia, North Otago, New Zealand.

3.5 Statistical analysis.

All statistical analysis in this research was analysed on 'Genstat 16'.

3.5.1 Experiment 1

All dry matter yield, botanical composition and leaf accumulation data in Experiment 1 were analysed using a one-way analysis of variance (ANOVA) in randomized blocks. Further analysis using a Fisher's protected LSD test (5%) was carried out on any results that showed a significant difference between means ($P < 0.05$).

Phyllochron data were analysed using a regression analysis. For each replicate, a simple linear regression was carried out between leaf accumulation (Y) and accumulated thermal time (X) (Tbase = 0°C). The coefficient of the regression line for each replicate was recorded. Phyllochron was calculated for each replicate using the following equation, where 'c' is the coefficient of the regression line.

$$\text{Phyllochron} = 1/c$$

Differences in phyllochron among cultivars were then detected using a one-way ANOVA. Split line regressions were also carried out between the mean leaf accumulation (Y) and thermal time (X) for each cultivar to quantify when each cultivar reached secondary leaf appearance.

The use of an arcsine transformation was considered for all percentage data (botanical composition). An arcsine transformation ensures data are normally distributed. An arcsine is necessary if percentage data falls outside of the following ranges: 0-30%, 30-70%, 70-100%. If data falls outside of these ranges, then the data may not be normally distributed. For all three experiments in this research, percentage data were within these ranges, with the exception of 1-2 data points in each data set that fell slightly outside. Because almost all data were within the ranges, data were normally distributed so no arcsine transformation was carried out (Sokal & Rohlf, 1981).

3.5.2 Experiment 2

All dry matter yield and botanical composition data from Experiment 2 were analysed using a one-way ANOVA in randomized blocks. Significant results were further analysed using Fisher's protected LSD test.

Data from Woolshed 5 were not statistically analysed because there was no experimental design, but were reported as observations.

3.5.3 Experiment 3

All height, botanical composition and dry matter yield data in Experiment 3 were analysed using a two-way ANOVA with a criss-cross design. Treatment levels were cultivar and GA level. Significant results were further analysed using Fisher's protected LSD test. Intercations between cultivar and GA level were presented in the figures.

4 RESULTS

4.1 Experiment 1 – Iversen Field (I3)

4.1.1 Emergence

Figure 4.1.1 shows there was no difference (P < 0.924) among cultivars in the number of established seedlings on the 21/04/2017, averaging 85.4 ± 10.5 /m² 35 days after sowing (DAS).

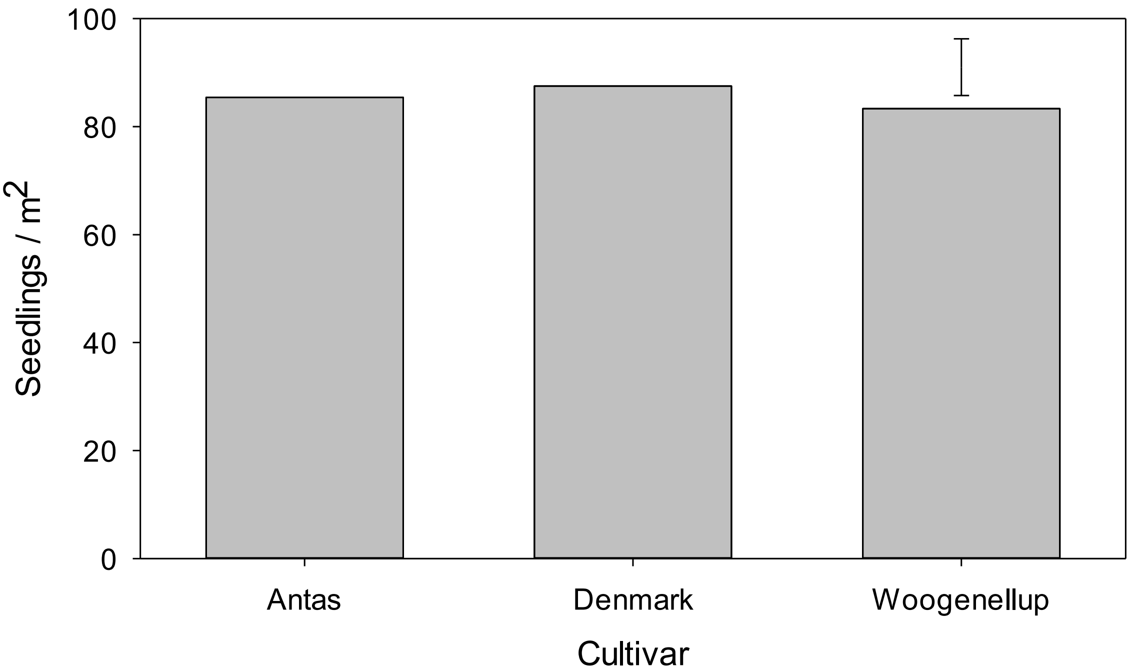

Figure 4.1.1: Number of seedlings/m² of three subterranean clover cultivars on the 21/04/2017 (35 DAS) at Lincoln University, Canterbury, New Zealand. Error bar indicates the standard error of the mean.

4.1.2 Leaf appearance

The final number of leaves on the marked plants did not differ (P < 0.154) among cultivars on the 08/09/2017 (175 DAS), and averaged 12.2 ± 3.17 leaves/ plant (Figure 4.1.2).

'Denmark' had a shorter (P < 0.025) mean phyllochron than 'Antas' or 'Woogenellup' from the 05/05/2017 to the 08/09/2017. 'Denmark' had an average phyllochron of 50.0 °Cd/leaf, compared with 97.9 and 116 °Cd/leaf for 'Woogenellup' and 'Antas', respectively.

Split line regressions showed ‘Antas’ to produce a leaf every 150 °Cd until leaf number 5, or up to 1058 °Cd, after which a leaf was produced every 53 °Cd (Figure 4.1.3). ‘Woogenellup’ produced a leaf every 93 °Cd until leaf number 5 (945 °Cd), after which a leaf was produced every 50 °Cd. ‘Denmark’ produced a leaf every 94 °Cd until leaf number 5 (828 °Cd), after which a leaf was produced every 36 °Cd. This implies that all these subterranean clover cultivars began secondary leaf production at leaf number 5.

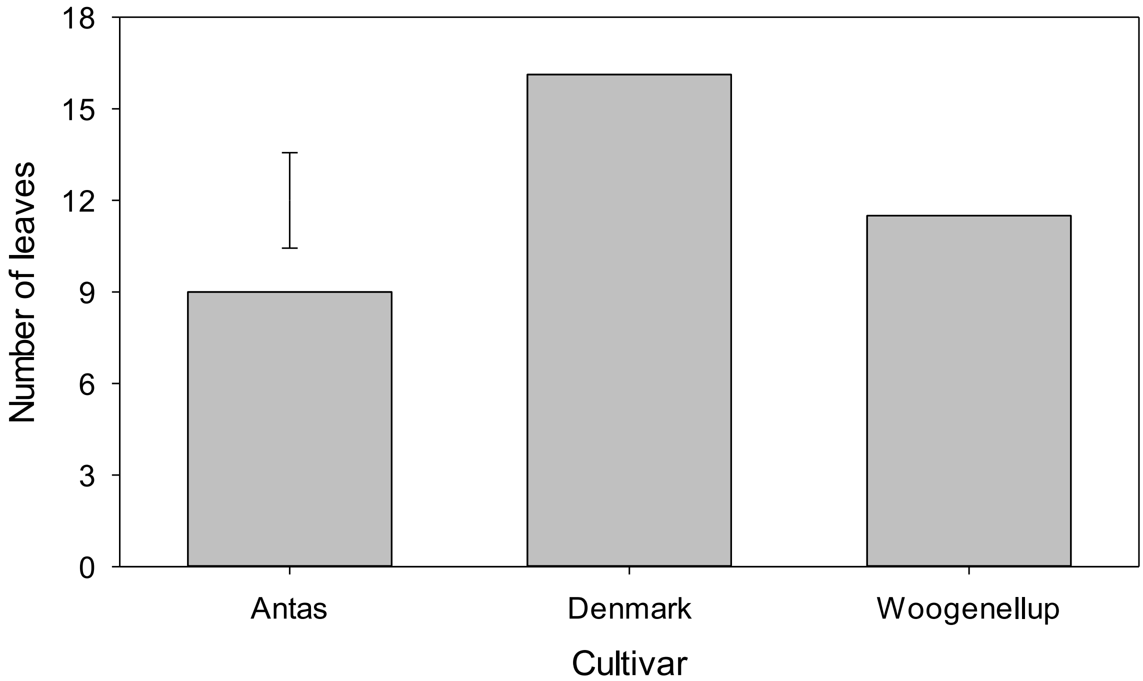

Figure 4.1.2: Final number of leaves on the 08/09/2017 of three subterranean clover cultivars over-drilled into established ryegrass pastures on the 17/03/2017 at Lincoln University, New Zealand. Error bar indicates the standard error of the mean.

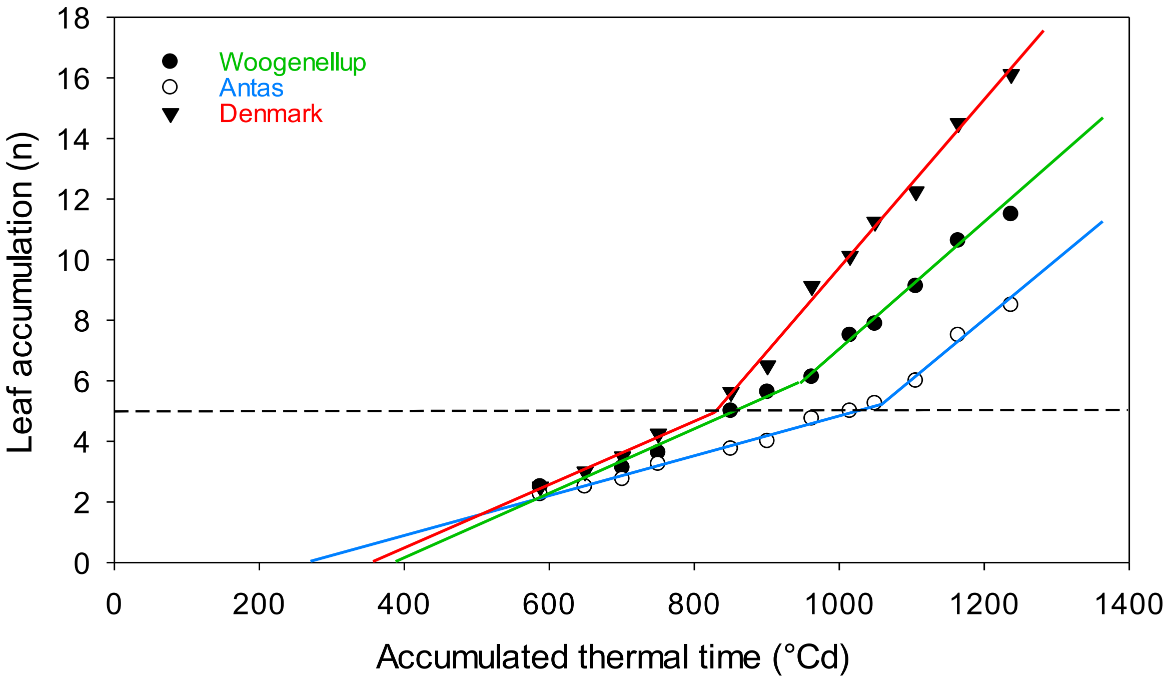

Figure 4.1.3: Number of leaves against accumulated thermal time (T base = 0 °C) of three subterranean clover cultivars over-drilled into established ryegrass pastures on the 17/03/2017 at Lincoln University, New Zealand. The dotted line represents leaf number 5.

Table 4.1: Coefficients for regression equations that describe the lines presented in Figure 4.1.3

Cultivar	Line 1 equation	Breakpoint	Line 2 gradient	r^2
Antas	y = 0.006683x - 1.8	(1058, 5.251)	y = 0.01879	0.992
S.E.M	0.000352		0.00177	
Woogenellup	y = 0.01076x - 4.2	(945, 5.94)	y = 0.01988	0.993
S.E.M	0.00102		0.00121	
Denamrk	y = 0.01055x - 3.8	(828, 4.966)	y = 0.027648	0.996
S.E.M	0.00252		0.000878	

4.1.3 Pasture yields.

At the final harvest on the 11/10/2017, 'Woogenellup' had greater ($P < 0.024$) dry matter yields than all other treatments. 'Woogenellup' yielded 1296 kg DM/ha, compared with 1008 kg, 987 kg, and 982 kg DM/ha for 'Antas', controls and 'Denmark', respectively (Figure 4.1.4).

Grass yields did not differ (P < 0.358) among treatments on the 11/10/2017. Grass yields averaged 875 ± 109 kg DM/ha across all treatments (Figure 4.1.5). Therefore, the differences in total yield were attributed to the clover content.

'Woogenellup' and 'Antas' had greater (P < 0.024) weed yields than control plots. 'Woogenellup' and 'Antas' had weed yields of 131 and 118 kg DM/ha, compared with 51.2 kg DM/ha for controls (Figure 4.1.6). 'Denmark' weed yields did not differ from other treatments, averaging 76.3 kg DM/ha.

When white and subterranean clover yields were combined, 'Woogenellup' had greater (P < 0.023) total clover yields compared with all other treatments. 'Woogenellup' had clover yields of 173 kg DM/ha, compared with 90 kg, 74 kg and 61 kg DM/ha for 'Antas', control and 'Denmark' treatments, respectively (Figure 4.1.7). Interestingly all clover in the 'Woogenellup', 'Antas' and 'Denmark' plots was subterranean clover, but the clover produced in control plots was all volunteer white clover.

Figure 4.1.4: Total dry matter yields (kg DM/ha) on the 11/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 17/03/2017 at Lincoln University, Canterbury, New Zealand. Error bar indicates the standard error of the mean. Different letters on the bars indicate a significant difference at α = 0.05.

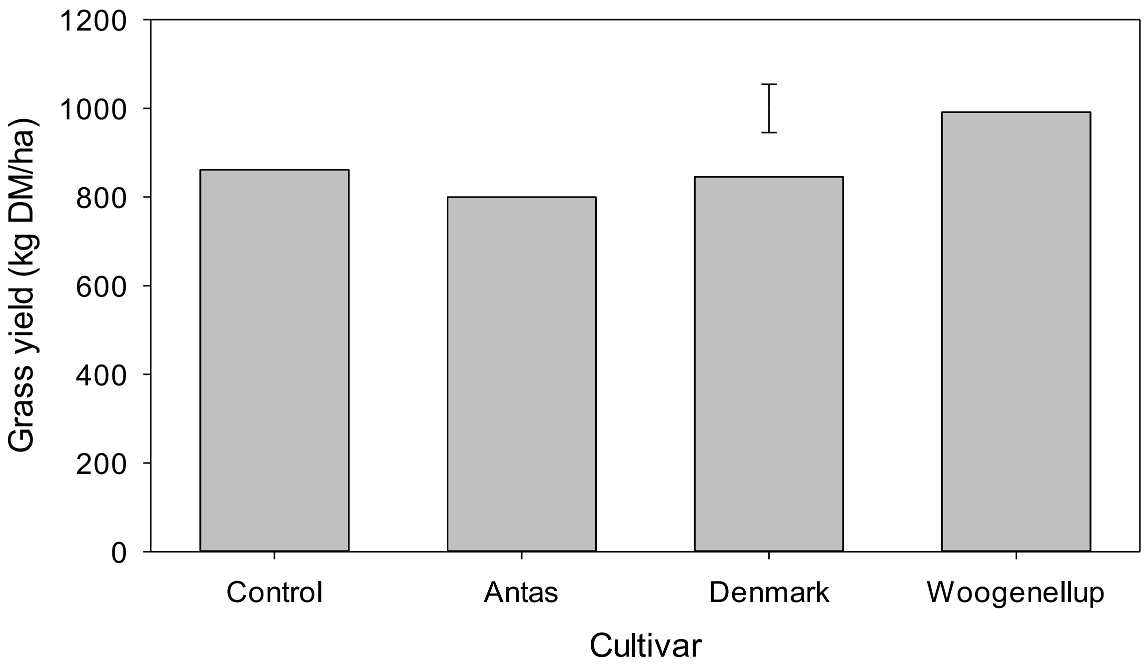

Figure 4.1.5: Grass yields (kg DM/ha) on the 11/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 17/03/2017 at Lincoln University, Canterbury, New Zealand. Error bar indicates the standard error of the mean.

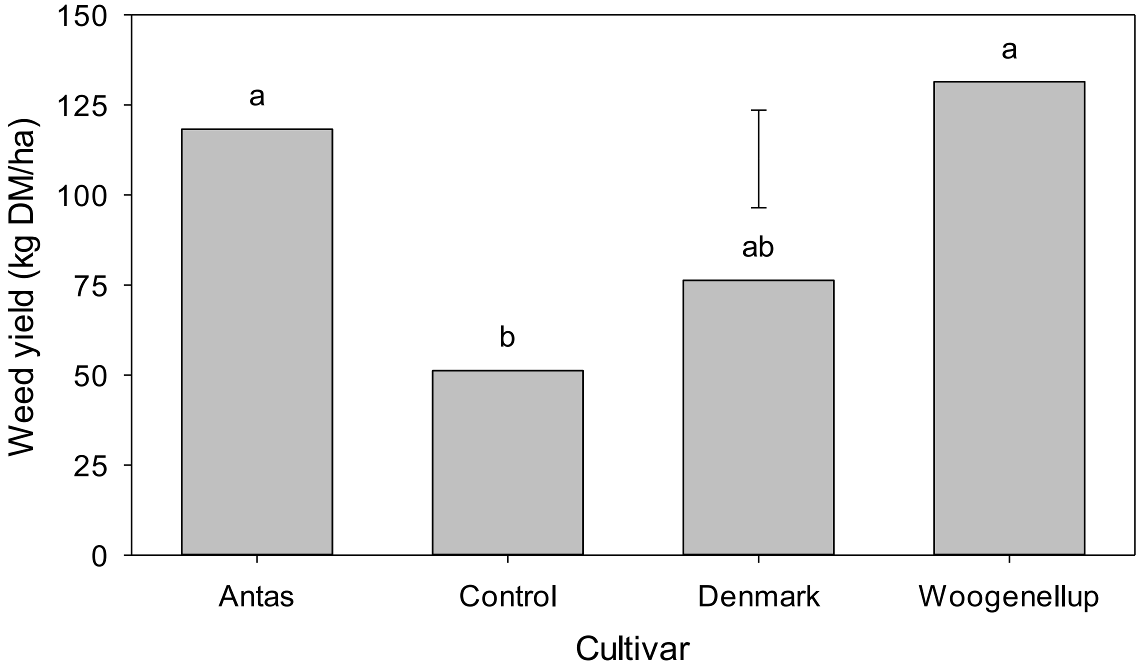

Figure 4.1.6: Weed yields (kg DM/ha) on the 11/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 17/03/2017 at Lincoln University, Canterbury, New Zealand. Error bar indicates the standard error of the mean. Different letters indicate a significant difference α = 0.05.

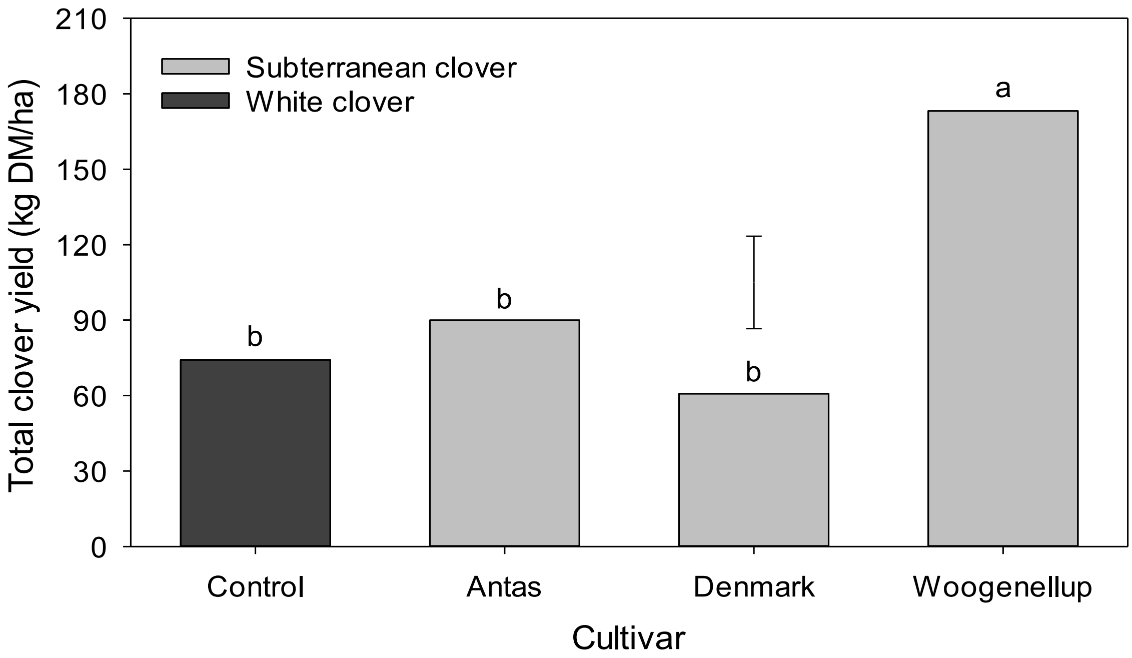

Figure 4.1.7: Total clover yields (kg DM/ha) on the 11/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 17/03/2017 at Lincoln University, Canterbury, New Zealand. Error bar indicates the standard error of the mean. Different letters on the bars indicate a significant difference α = 0.05.

4.1.4 Botanical composition.

There was an indication ($P < 0.074$) that 'Woogenellup' pastures had lower grass content than other pastures on the 11/10/2017. 'Woogenellup' pastures contained 75.8% grass, compared with 80.7%, 85.6% and 86.4% for 'Antas', 'Denmark' and controls, respectively (Table 4.1.2).

Control pastures had an average white clover content of 8.18%. 'Denmark', 'Antas' and 'Woogenellup' did not grow any white clover (Table 4.1.2). However, there were patches of pure white clover observed covering <5% of the experimental area. These were avoided in all plots during sampling.

'Woogenellup' pastures had higher ($P < 0.041$) subterranean clover content than 'Antas' and 'Denmark' pastures. 'Woogenellup' pastures had a subterranean clover content of 13.6%, compared with 7.90% and 6.30% for 'Antas' and 'Denmark', respectively (Table

4.1.2). Control pastures did not produce any subterranean clover, which shows there was no resident subterranean clover.

The weed content of pastures did not differ (P < 0.114) between treatments. Weed content averaged 8.84 ± 5.32% across all pastures (Table 4.1.2).

Table 4.2: Botanical composition (%) on the 11/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 17/03/2017 at Lincoln University, New Zealand.

Cultivar	Botanical composition (%)			
	Grass	White clover	Sub clover	Weeds
Control	86.4	8.18		5.38
Antas	80.7	0.00	7.89 b	11.4
Denmark	85.6	0.00	6.33 b	8.04
Woogenellup	75.8	0.00	13.6 a	10.6
S.E.M	4.30	-	2.74	2.58

Note: Different letter subscripts within a column indicate a significant difference at α = 0.05.

Plate 4-1: 'Woogenellup' subterranean clover on the 31/10/2017 at Experiment 1, Lincoln University, New Zealand.

4.2 Experiment 2 – Invernia.

4.2.1 Emergence.

Seedling establishment did not differ (P < 0.344) among cultivars on the 19/03/2017 at Experiment 2 in North Otago. Average seedlings/m² was 158 ± 30.1 /m² across all cultivars when measured 14 DAS (Figure 4.2.1). Based on the thousand seed weights (Section 3.1.3), more seedlings were established than there were seeds sown. This is likely due to inaccuracies in the calibration of the drill used at sowing, so a higher establishment rate than targeted was achieved.

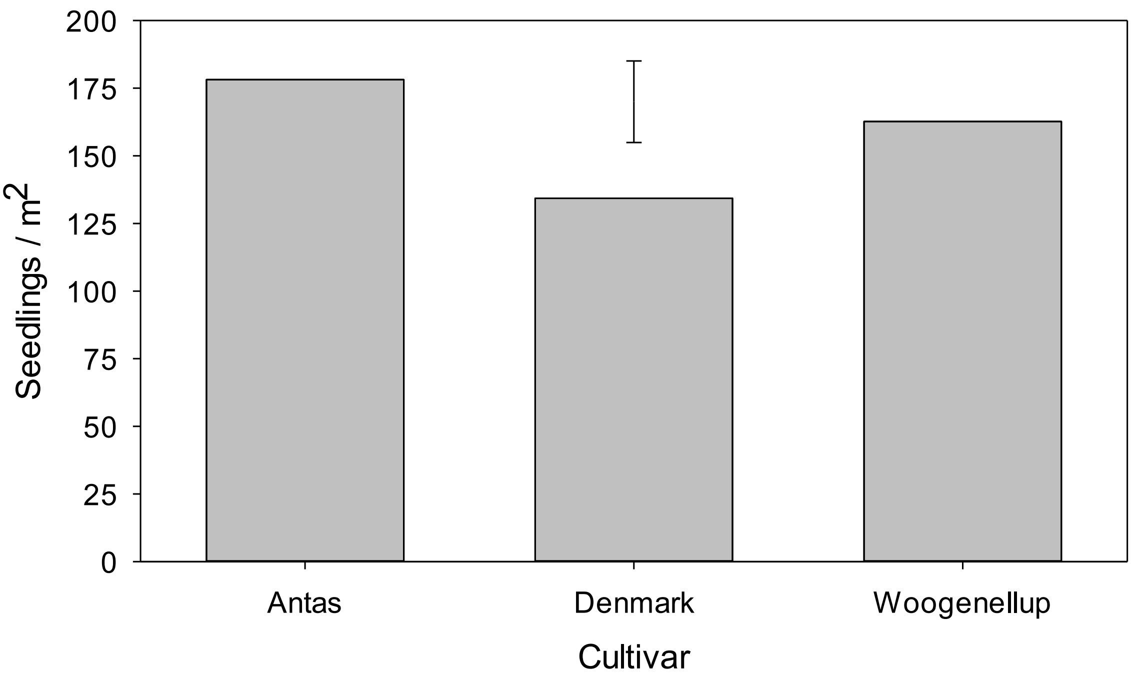

Figure 4.2.1: Establishment of three subterranean clover cultivars on the 19/03/2017 (14 DAS) at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean.

4.2.2 Pasture yields.

4.2.2.1 Airport 6.

Dry matter yields did not differ (P < 0.337) among treatments on the 03/10/2017, and averaged 1357 ± 121 kg DM/ha across all plots (Figure 4.2.2).

There was a trend ($P < 0.083$) for control treatments to have higher grass yields than those over-drilled with subterranean clover on the 03/10/2017. Control plots averaged 1316 kg grass DM/ha, compared with 1080, 991 and 978 kg grass DM/ha for 'Denmark', 'Antas' and 'Woogenellup' treatments, respectively (Figure 4.2.3).

There was no difference ($P < 0.415$) in the white clover yields among treatments on the 03/10/2017, averaging 80 ± 25.0 kg DM/ha (Figure 4.2.4).

As expected, control treatments produced no subterranean clover. 'Denmark' treatments also had less ($P < 0.005$) subterranean clover than 'Woogenellup' or 'Antas' on the 03/10/2017. 'Denmark' grew 54.4 kg DM/ha, compared with 189 and 168 kg DM/ha for 'Antas' and 'Woogenellup', respectively (Figure 4.2.5).

There was a trend ($P < 0.056$) towards lower weed yields in 'Denmark' and 'Control' plots on the 03/10/2017. Weed yields were 55 kg DM/ha for control and 50 kg DM/ha for and 'Denmark' plots, compared with 123 kg DM/ha for 'Antas' and 104 kg DM/ha for 'Woogenellup' (Figure 4.2.6).

'Woogenellup' and 'Antas' produced greater ($P < 0.002$) total clover yields than 'Denmark' and control treatments on the 03/10/2017. 'Woogenellup' and 'Antas' grew 297 and 239 kg clover DM/ha, compared with 105 and 90 kg clover DM/ha for 'Denmark' and control treatments, respectively (Figure 4.2.7).

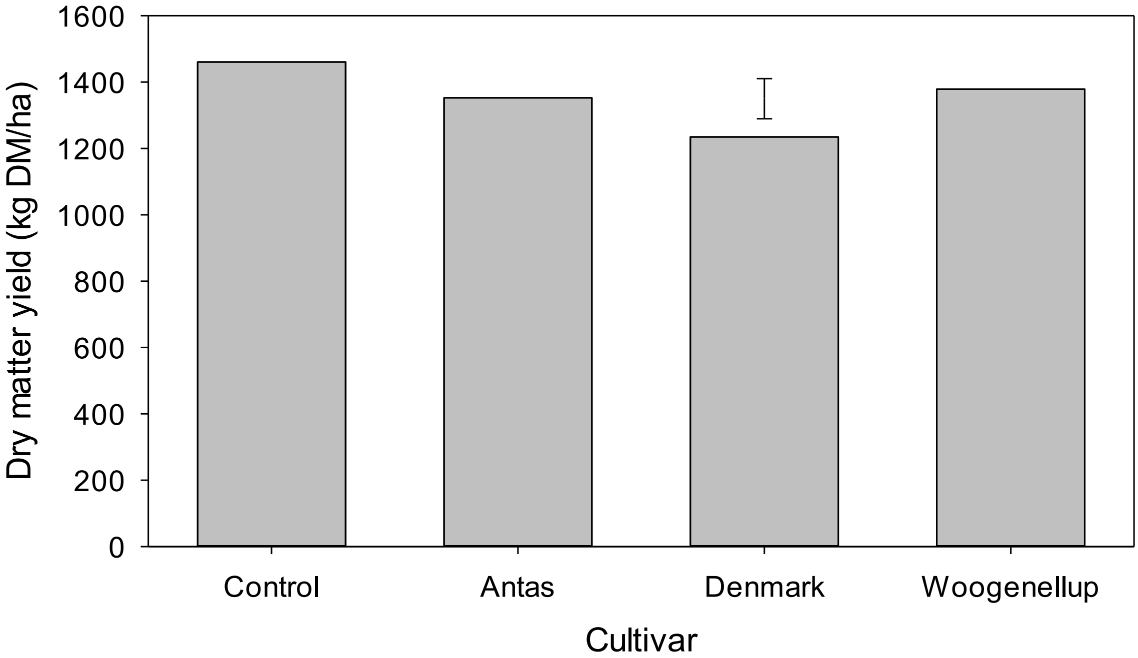

Figure 4.2.2: Dry matter yields on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean.

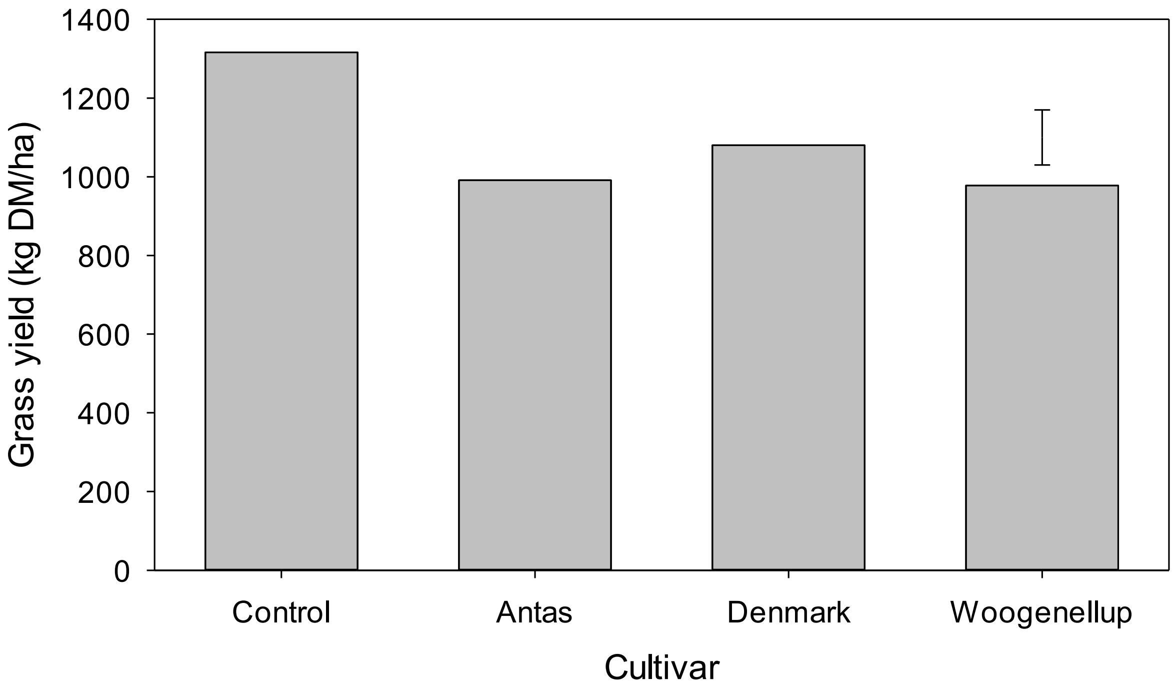

Figure 4.2.3: Grass yields on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean.

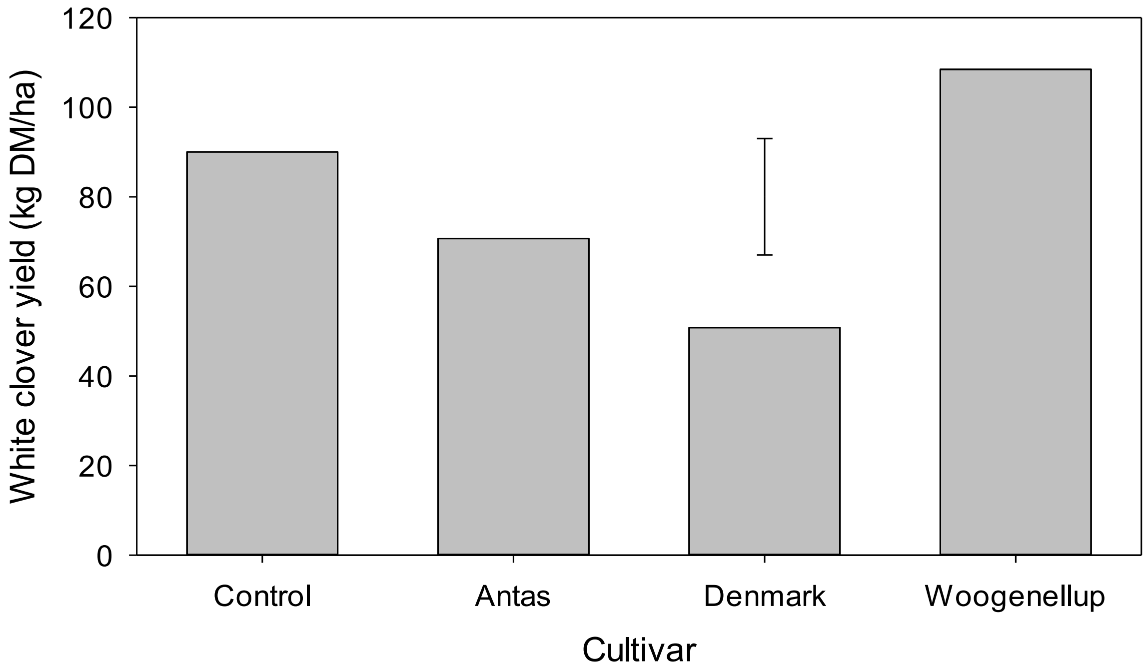

Figure 4.2.4: White clover yields on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean

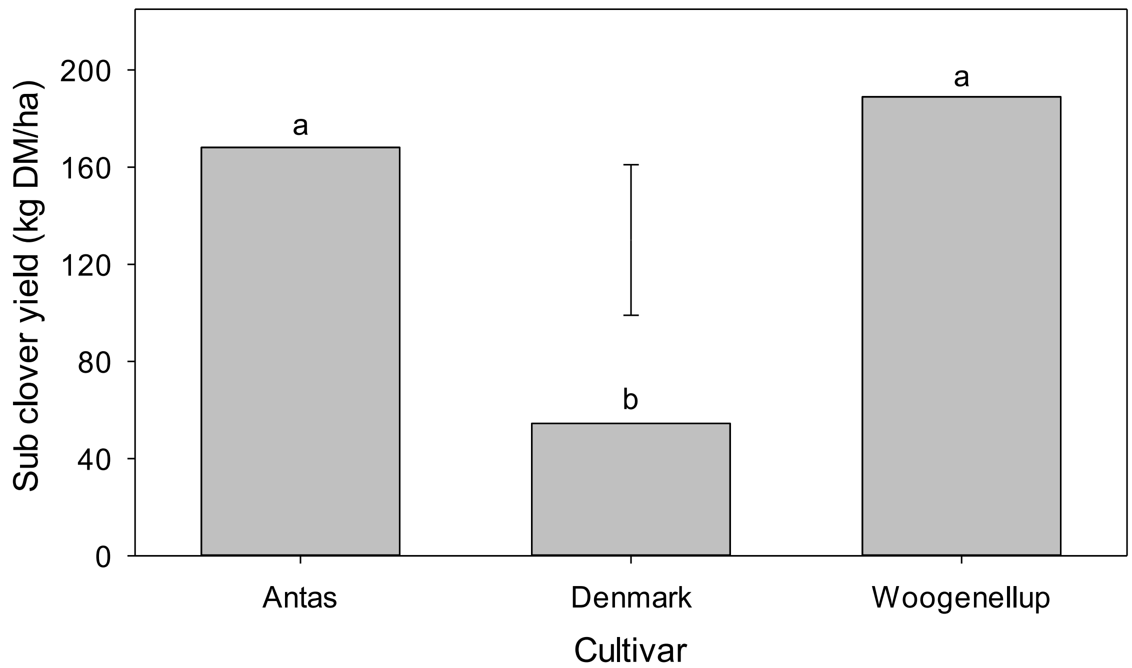

Figure 4.2.5: Subterranean clover yields on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean. Different letters indicate significant differences at α = 0.05.

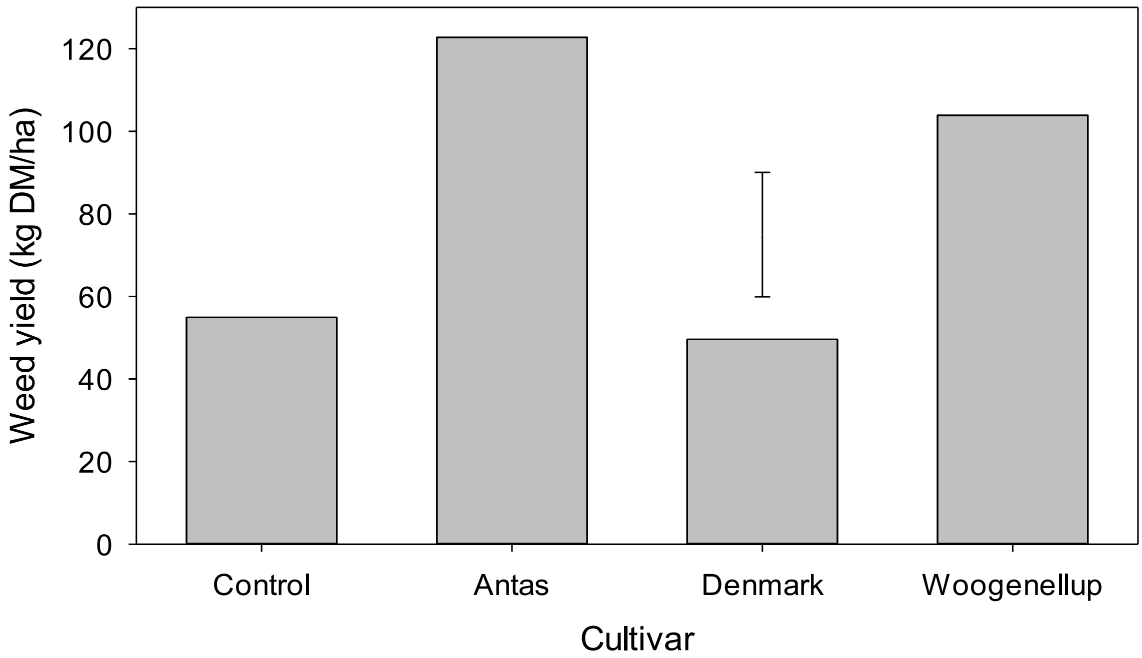

Figure 4.2.6: Weed yields on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean.

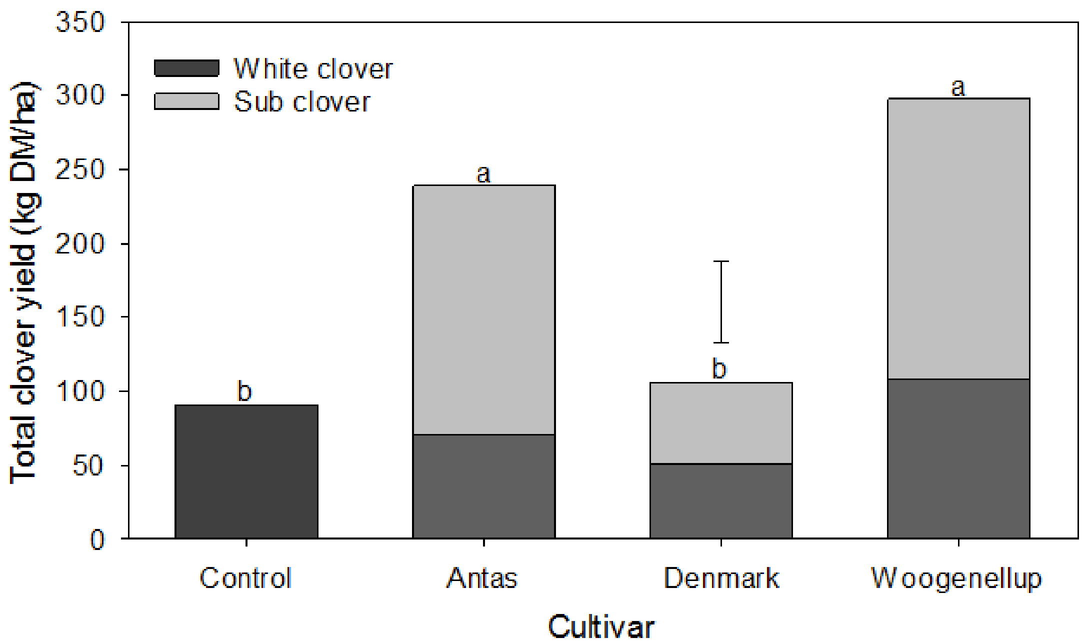

Figure 4.2.7: Total clover yields on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand. Error bar indicates the standard error of the mean. Different letters indicate significant differences at α = 0.05.

4.2.2.2 Botanical composition

The percentage of grass in the pasture was greater (P < 0.001) in 'Denmark' and control pastures, compared with 'Woogenellup' and 'Antas' on the 03/10/2017 (Table 4.2.1). Control and 'Denmark' plots averaged 89.9% and 86.5% grass, compared with 73.4% and 70.6% for 'Antas' and 'Woogenellup', respectively.

White clover content did not differ (P < 0.596) among treatments, and averaged 5.97% ± 2.59 (Table 4.2.1).

There was a trend (P < 0.080) for 'Denmark' pastures to have lower subterranean clover content than 'Antas' or 'Woogenellup'. 'Denmark' had a mean sub clover content of 4.70%, compared with 11.6% and 13.9% for 'Antas' and 'Woogenellup', respectively (Table 4.2.1).

The weed content of pastures did not differ (P < 0.117) among treatments, with 6.40% ± 2.48 (Table 4.2.1).

'Woogenellup' and 'Antas' had greater (P < 0.001) total clover content than 'Denmark' or control pastures. Clover content was 21.7% and 17.1% for 'Woogenellup' and 'Antas', compared with 9.22% and 5.95% for 'Denmark' and controls, respectively (Table 4.2.1).

Table 4.3: Botanical composition (%) on the 03/10/2017 of established perennial ryegrass pastures over-drilled with three subterranean clover cultivars on the 05/03/2017 at Invernia, North Otago, New Zealand.

	Botanical composition (%)				
Cultivar	Grass	White clover	Sub clover	Weeds	Total clover
Control	89.8 a	5.95		4.20	5.95 b
Antas	73.4 b	5.58	11.5	9.41	17.1 a
Denmark	86.4 a	4.48	4.74	4.30	9.22 b
Woogenellup	70.5 b	7.87	13.8	7.69	21.7 a
S.E.M	5.05	2.49	3.93	2.48	3.74

Note: Different letter subscripts within a column indicate a significant difference at α = 0.05.

4.2.3 Woolshed 5

4.2.3.1 Herbage yield

The components of yield of the three blocks in Woolshed 5 are shown in Table 4.2.2. Grass yields averaged 972, 875 and 935 kg DM/ha for the ‘bottom’, ‘middle’ and ‘top’ blocks, respectively. White clover yield averaged 330 kg DM/ha for the ‘middle’ block, compared with 4.21 kg DM/ha and none for the ‘bottom’ and ‘top’ blocks, respectively. In contrast, subterranean clover yields averaged 252 and 320 kg DM/ha for the ‘bottom’ and ‘top’ blocks, respectively. Weed yields averaged 37.6, 56.6 and 11.2 kg DM/ha for the ‘bottom’, ‘middle’ and ‘top’ blocks, respectively.

Table 4.4: Herbage yield (kg DM/ha) on the 09/09/2017 of an established perennial ryegrass pasture over-drilled with subterranean clover on the 05/03/2017 at Invernia, North Otago, New Zealand.

Block	Herbage yield (kg DM/ha)			
	Grass	White clover	Sub clover	Weeds
Bottom	972	4.21	252	37.6
Middle	875	330	5.05	56.6
Top	935	0.00	320	11.2

Plate 4-2: ‘Woogenellup’ subterranean clover in the ‘top’ block of Woolshed 5 on the 03/09/2017 at Invernia, North Otago, New Zealand.

4.2.3.2 Botanical Composition.

The botanical composition of three blocks in Woolshed 5 is shown in Table 4.2.3. Grass content averaged 76.8%, 69.1% and 73.8% for the 'bottom', 'middle' and 'top' blocks, respectively. The 'middle' block had a white clover content of 26.1, compared with 0.33% and none for the 'bottom' and 'top' blocks, respectively. In contrast, the 'middle' block had little subterranean clover <1%, compared with 19.9% and 25.3% for the 'bottom' and 'top' blocks, respectively. Weed content was <5% in all blocks.

Table 4.5: Botanical composition (%) on the 09/09/2017 of an established perennial ryegrass pasture over-drilled with subterranean clover on the 05/03/2017 at Invernia, North Otago, New Zealand.

Block	Botanical composition (%)			
	Grass	White clover	Sub clover	Weeds
Bottom	76.8	0.33	19.9	2.97
Middle	69.1	26.1	0.40	4.47
Top	73.8	0.00	25.3	0.88

4.3 Experiment 3 – Iversen Field (I2) – Gibberellic acid on subterranean clover.

4.3.1 Plant height.

4.3.1.1 Measurement 1 – 13/09/2017

Subterranean clover that recieved GA (13.3 cm) was taller ($P < 0.005$) than control plants (10.3 cm) on the 13/09/2017 (Figure 4.3.1). There was no difference ($P < 0.156$) in the height of different cultivars (11.6 ± 2.29 cm), and no interaction ($P < 0.783$) between cultivar and GA level.

4.3.1.2 Measurement 2 – 06/10/2017.

By the second measurement date, plant height did not differ ($P < 0.716$) as a result of GA application (6/10/2017), and averaged 27.7 ± 1.40 cm (Figure 4.3.2). There was also no difference ($P < 0.273$) in height among cultivars, and no interaction ($P < 0.401$) between GA application and cultivar.

Plate 4-3: 'Leura' subterranean clover shown with a height stick on the 13/09/2017 at Experiment 3, Lincoln University, New Zealand.

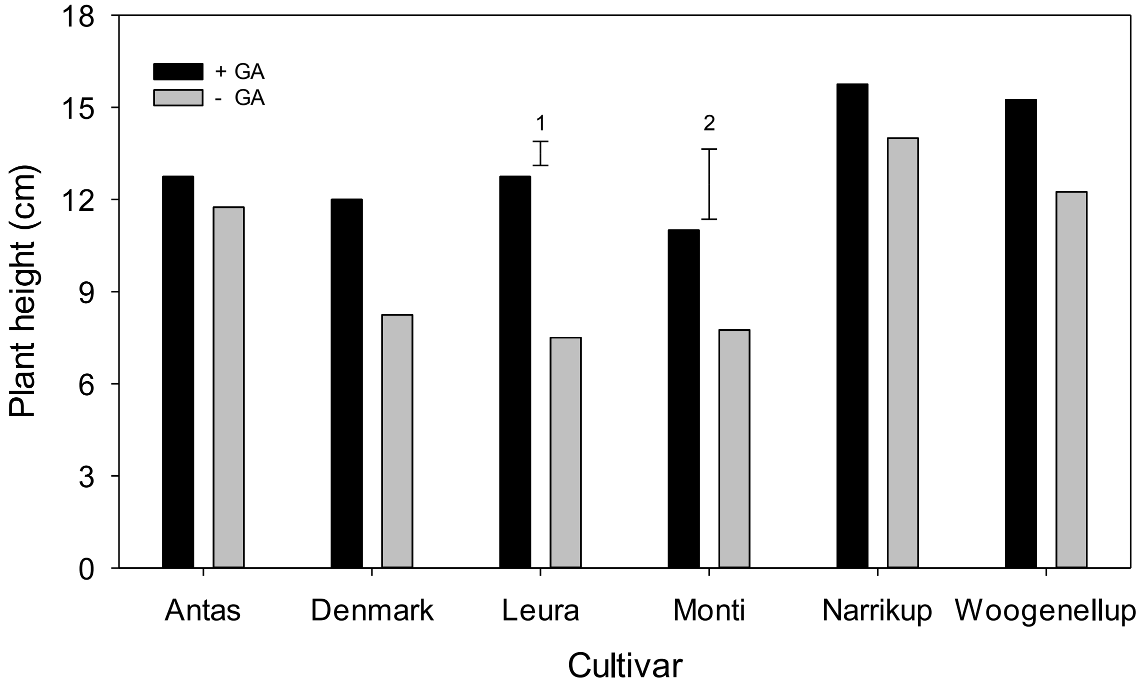

Figure 4.3.1: Height of six subterranean clover cultivars on the 13/09/2017 with (+) or without (-) GA, applied on the 31/07/2017, at Lincoln University, New Zealand. Error bars indicate the standard error of the mean between cultivars (1) and GA level (2).

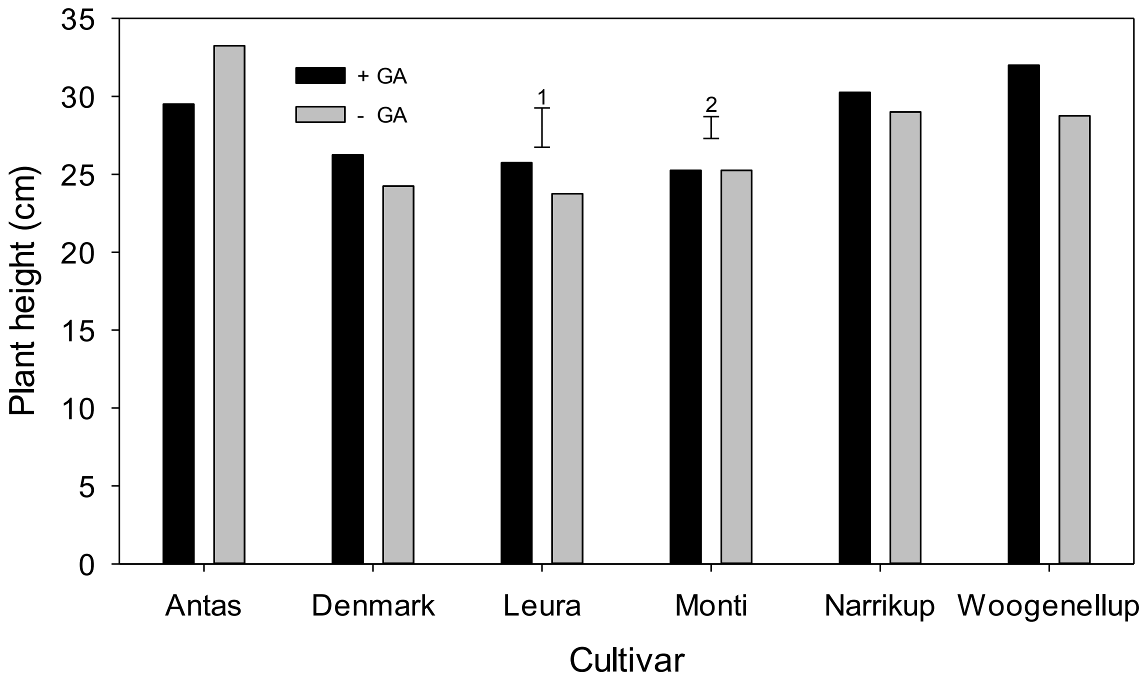

Figure 4.3.2: Height of six subterranean clover cultivars on the 06/10/2017 with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

4.3.2 Pasture cuts

4.3.2.1 Dry matter yields - 1st cut (16th August 2017).

Dry matter yield did not differ ($P < 0.498$) among cultivars on the 16/08/2017. Dry matter yield averaged 501 ± 112 kg DM/ha across all cultivars (Figure 4.3.3), but did not differ ($P < 0.128$) with the application of GA, or the interaction ($P < 0.321$) between cultivars and GA level.

Subterranean clover yield did not differ ($P < 0.234$) among cultivars. Sub clover yield averaged 387 ± 91.8 kg DM/ha across all cultivars (Figure 4.3.4), but did not differ ($P < 0.142$) with the application of GA or the interaction ($P < 0.876$).

Weed yield did not differ ($P < 0.761$) among cultivars, and averaged 114 ± 46.0 kg DM/ha (Figure 4.3.5). It also did not differ ($P < 0.775$) with the application of GA, or the interaction ($P < 0.116$).

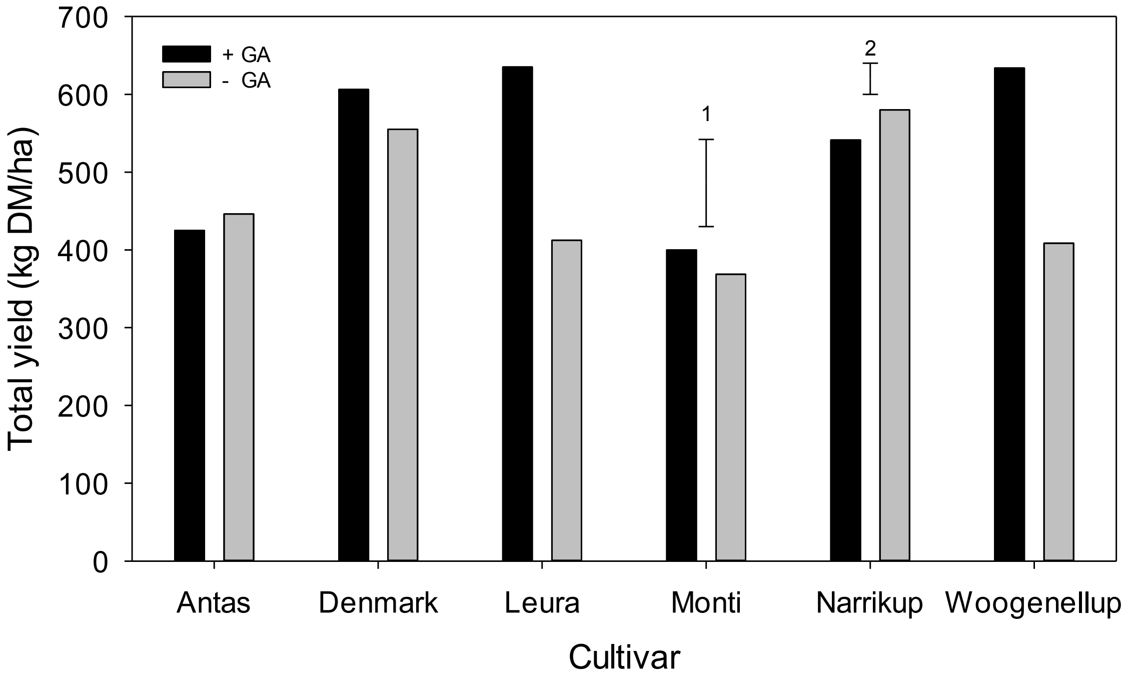

Figure 4.3.3: Total yield (kg DM/ha) on the 16/08/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

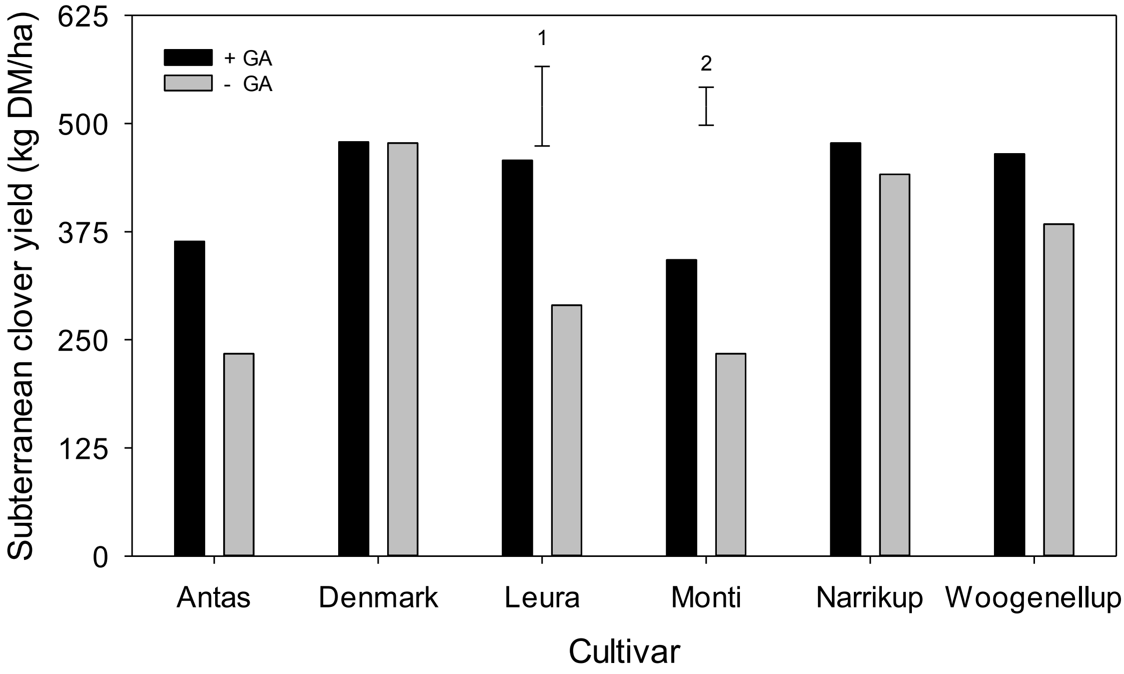

Figure 4.3.4: Subterranean clover yield (kg DM/ha) on the 16/08/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

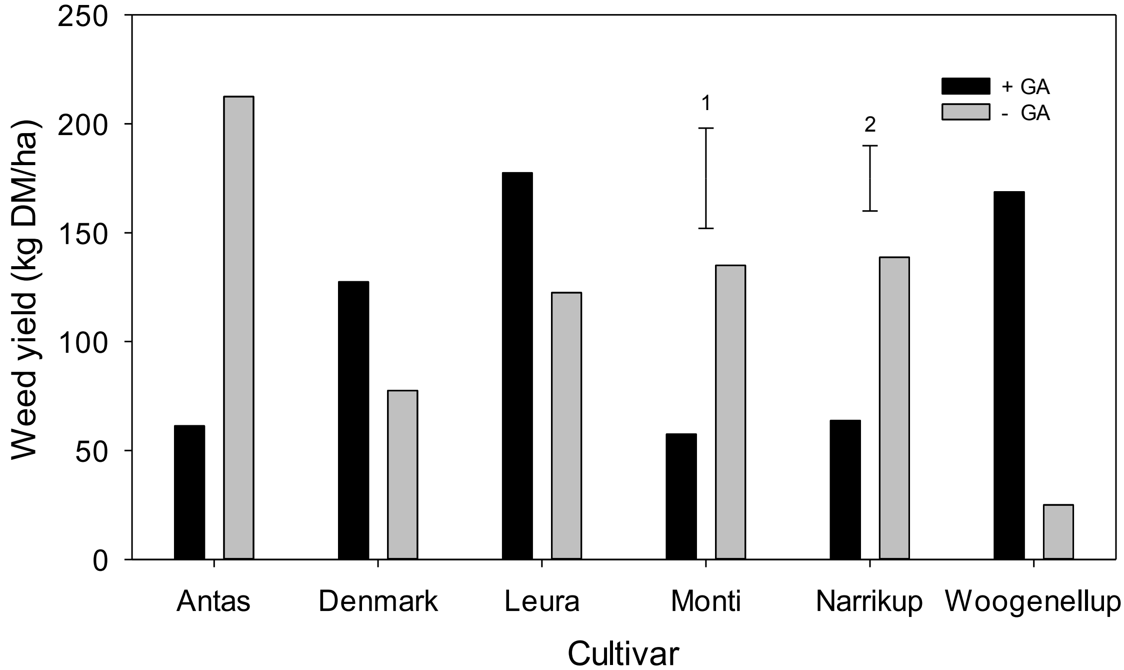

Figure 4.3.5: Weed yield (kg DM/ha) on the 16/08/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

4.3.2.2 Botanical Composition - 1st cut (16th August 2017).

Subterranean clover content did not differ (P < 0.166) among cultivars on the 16/08/2017. Sub clover content averaged 75.8 ± 8.70% across all cultivars and GA levels (Table 4.3.1), and there was no interaction (P < 0.251).

Weed content did not differ (P < 0.166) among cultivars, averaged 24.2 ± 8.70% across all cultivars (Table 4.3.1). Weed content did not differ (P < 0.453) with the application of GA, averaging 24.2 ± 6.14% across both GA levels, and there was no interaction (P < 0.453).

Table 4.6: Botanical composition (%) on the 16/08/2017 of pure subterranean clover, with (+) or without (-) GA applied on the 31/07/2017 at Lincoln University, New Zealand.

Cultivar	Weeds		Sub clover	
	- GA	+ GA	- GA	+ GA
Antas	53.9	70.5	46.1	29.5
Denmark	86.7	79.7	13.3	20.3
Leura	68.7	72.1	31.3	27.9
Monti	66.3	85.1	33.7	14.9
Narrikup	71.7	87.3	28.3	12.7
Woogenellup	91.6	76.1	8.35	23.9
S.E.M	8.70			

4.3.2.3 Dry matter yields - 2nd cut (13th September).

Total yields did not differ (P < 0.163) among cultivars at the second cut on the 13/09/2017. Total yield averaged 1555 ± 248 kg DM/ha among all cultivars and GA levels.

Subterranean clover yields differed (P < 0.009) among cultivars. 'Narrikup' (1,741 kg DM/ha) grew more sub clover than all other cultivars except 'Woogenellup' (1,321 kg DM/ha). 'Woogenellup' grew more sub clover than 'Monti' (887 kg DM/ha), but did not differ from 'Denmark' (1,248 kg DM/ha), 'Antas' (1,080 kg DM/ha) or 'Leura' (1,019 kg DM/ha) (Figure 4.3.7). Subterranean clover yields did not differ (P < 0.103) with the application of GA, averaging 1,216 ± 115 kg DM/ha, and there was no interaction (P < 0.137) between cultivar and GA level.

Weed yields did not differ (P < 0.207) among cultivars. Weed yield averaged 339 ± 115 kg DM/ha across all cultivars (Figure 4.3.8), and was not affected (P < 0.774) by the application of GA, or the interaction (P < 0.800).

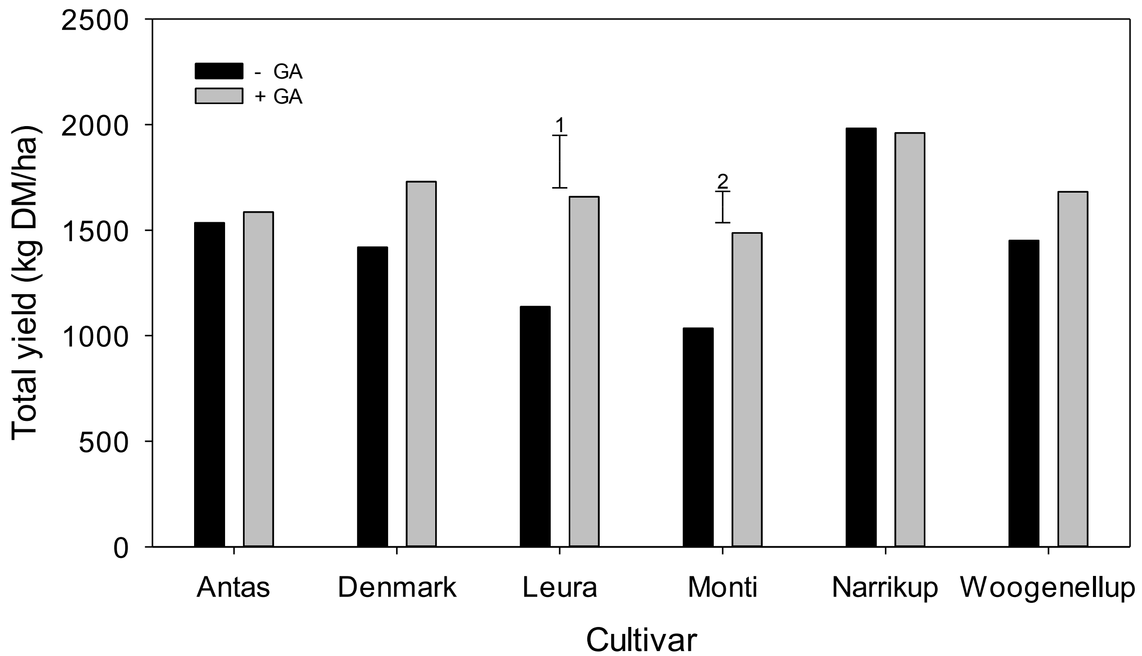

Figure 4.3.6: Total yield (kg DM/ha) on the 13/09/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

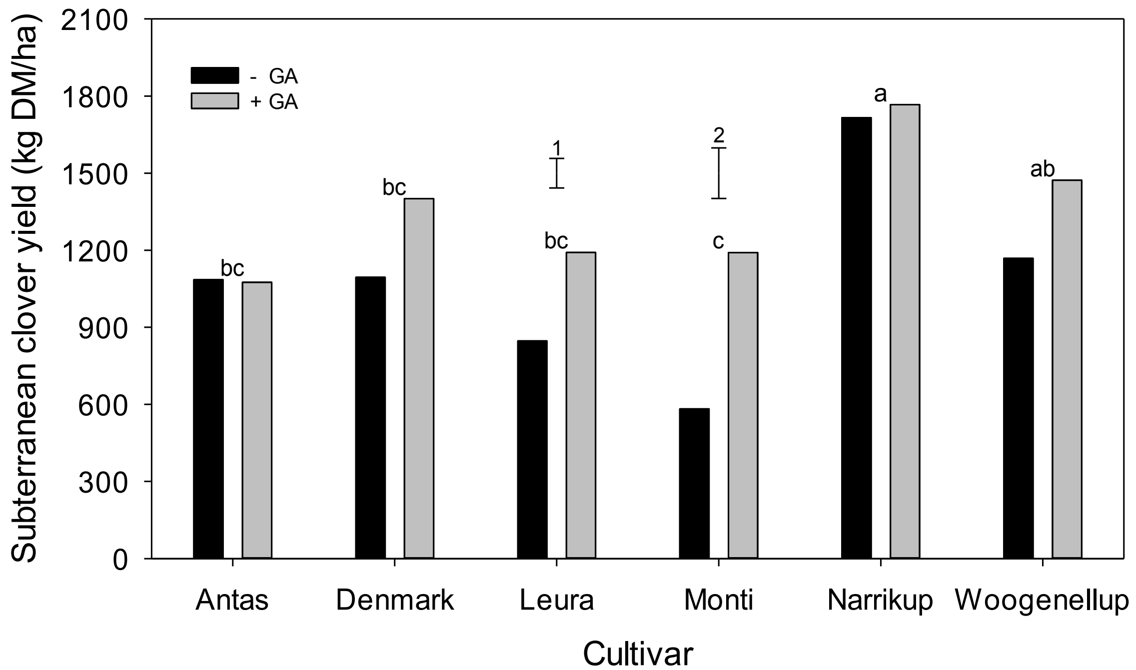

Figure 4.3.7: Subterranean clover yield (kg DM/ha) on the 13/09/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2). Different letters indicate a significant difference in yield between cultivars at α = 0.05.

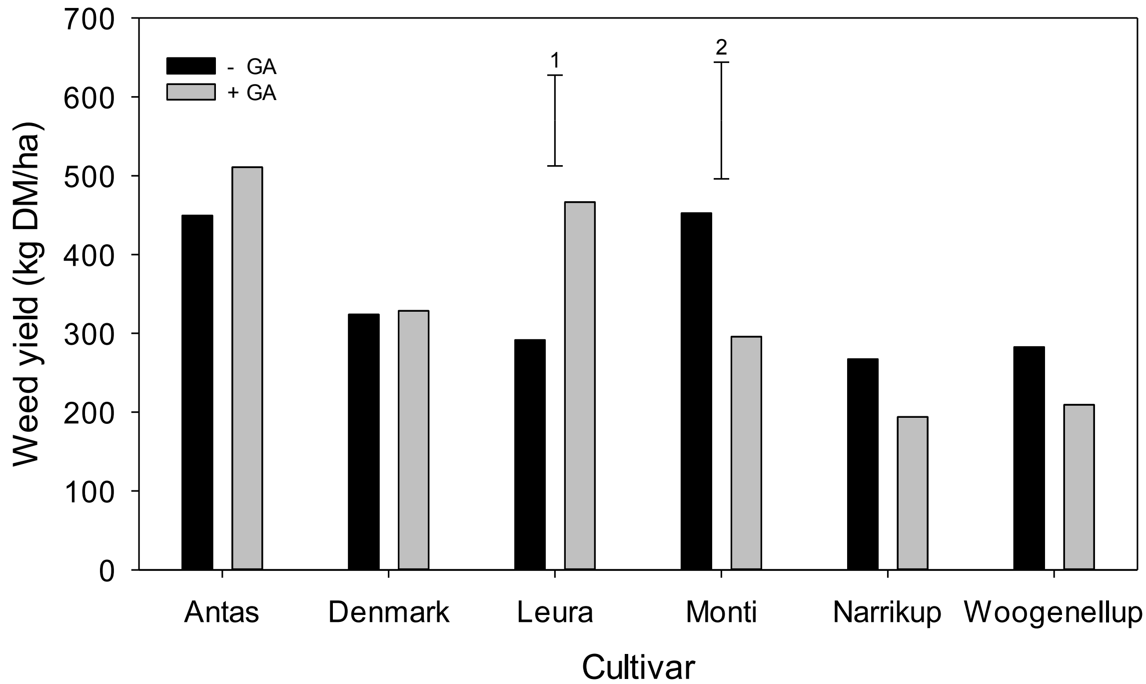

Figure 4.3.8: Weed yield (kg DM/ha) on the 13/09/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

4.3.2.4 Botanical composition - 2nd cut (13th September).

Subterranean clover content differed (P < 0.014) among cultivars on the 13/09/2017. ‘Narrikup’ (88.5%) had a greater sub clover content than ‘Leura’ (73.0%), ‘Monti’ (69.6%) and ‘Antas’ (67.6%) (Table 4.7). ‘Woogenellup’ (85.4%) had a greater sub clover content than ‘Monti’ and ‘Antas’, but was not different to ‘Narrikup’, ‘Denmark’ (78.4%) or ‘Leura’. Sub clover content also differed (P < 0.047) between GA levels. Plots that received GA on the 31/07/2017 averaged 79.7% sub clover, compared with 74.4% for those that received no GA.

Weed content differed (P < 0.014) among cultivars. ‘Antas’ (32.3%) and ‘Monti’ (30.4%) had greater weed content than ‘Woogenellup’ (14.7%) and ‘Narrikup’ (11.5%) (Table 4.3.2). ‘Leura’ (30.4%) had greater weed content than ‘Narrikup’, but was not different to any other treatment. Weed content differed (P < 0.047) between GA levels. Plots that received GA on the 31/07/2017 averaged 20.3% weeds compared with 25.6% for plots that received no GA.

Table 4.7: Botanical composition on the 16/08/2017 of pure subterranean clover at Lincoln University, New Zealand.

Cultivar	Sub clover content (%)	Weed content (%)
Antas	67.6 c	32.4 a
Denmark	78.4 abc	21.6 abc
Leura	73.0 bc	27.0 ab
Monti	69.6 c	30.4 a
Narrikup	88.5 a	11.5 c
Woogenellup	85.3 ab	14.7 bc
S.E.M	5.88	

Note: Different letter subscripts within a column indicate a significant difference at α = 0.05.

4.3.2.5 Dry matter yields - 3rd cut (6th October)

Total yields did not differ (P < 0.217) among cultivars on the 06/10/2017. Total yield averaged 3510 ± 473 kg DM/ha across all cultivars (Figure 4.3.9), but did not differ (P < 0.278) between GA levels, and there was no interaction (P < 0.338).

There was a trend (P < 0.083) for 'Monti' to have lower subterranean clover yields than other cultivars. 'Monti' produced 2170 kg DM/ha, compared with 3680, 3600, 3530, 2740 and 2690 kg DM/ha for 'Woogenellup', 'Antas', 'Narrikup', 'Leura' and 'Denmark', respectively (Figure 4.3.10). Sub clover yield did not differ (P < 0.537) between GA levels, and there was no interaction (P < 0.626).

Weed yield did not differ (P < 0.157) among cultivars, averaging 441 ± 243 kg DM/ha (Figure 4.3.11). It also did not differ (P < 0.511) between GA levels, and there was no interaction (P < 0.514).

Growth rate (°Cd/kg DM) of subterranean clover did not differ (P < 0.152) among cultivars or GA levels from the 16/08/2017 to the 06/10/2017. Growth rate averaged 6.36 ± 0.882 kg DM/°Cd across all cultivars.

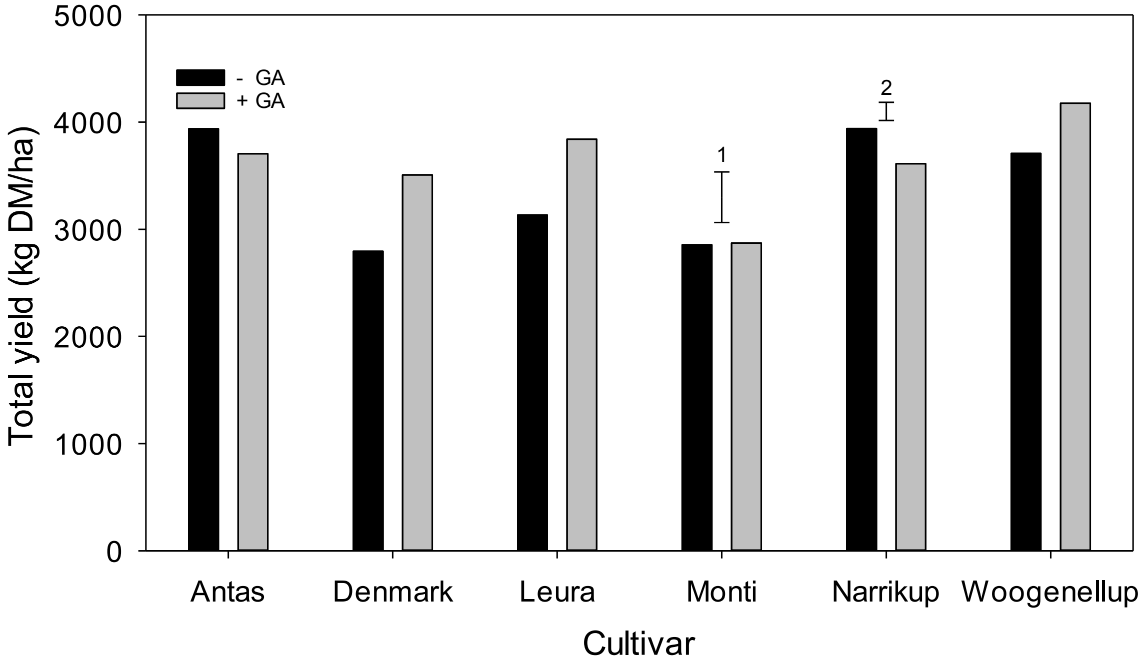

Figure 4.3.9: Total yield (kg DM/ha) on the 06/10/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

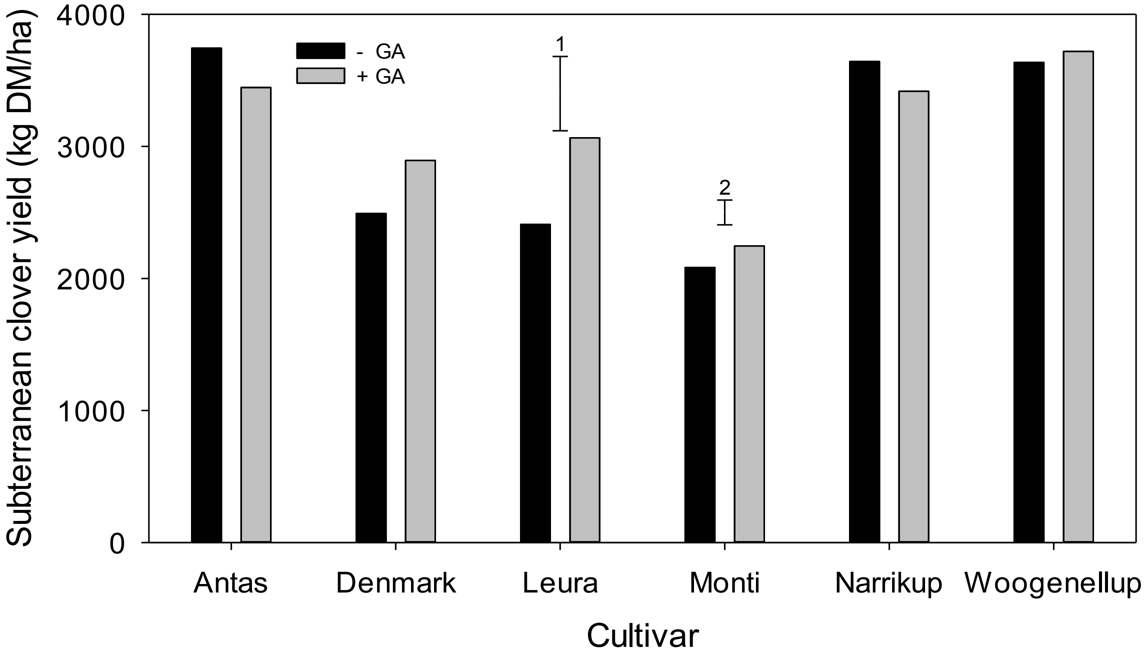

Figure 4.3.10: Subterranean clover yield (kg DM/ha) on the 06/10/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

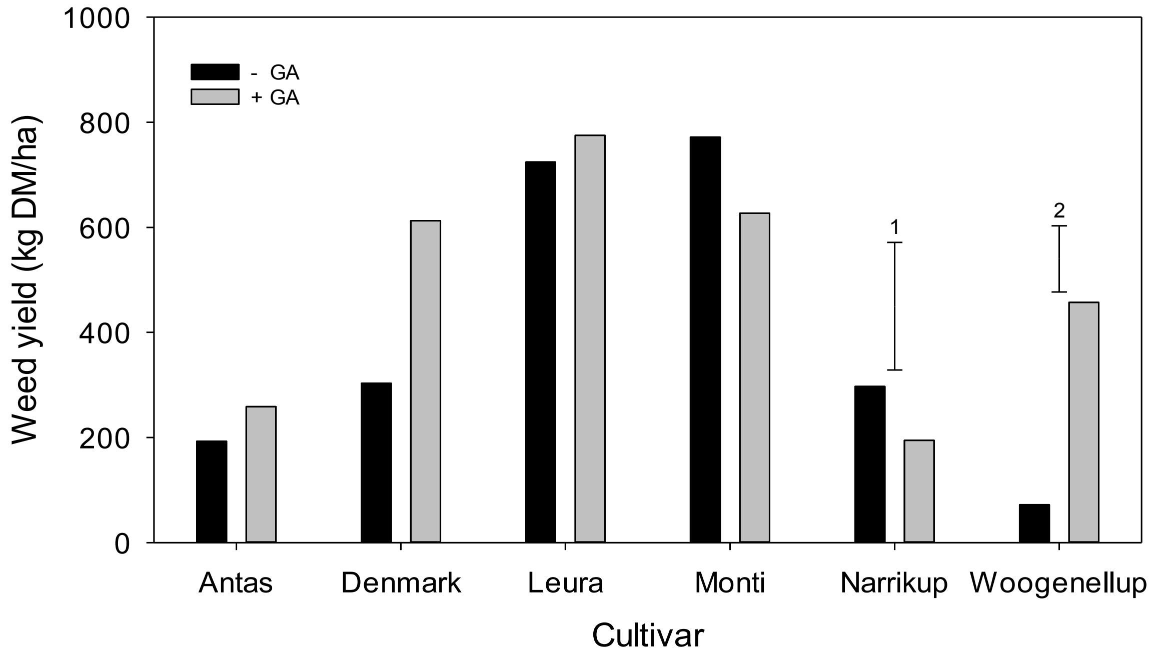

Figure 4.3.11: Weed yield (kg DM/ha) on the 06/10/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand. Error bars indicate the standard error of the mean among cultivars (1) and GA level (2).

Plate 4-4: 'Narrikup' subterranean clover on 06/10/2017 at Experiment 3, Lincoln University, New Zealand.

4.3.2.6 Botanical composition - 3rd cut (6th October).

Subterranean clover content did not differ ($P < 0.143$) among cultivars on the 06/10/2017, and averaged 86.5 ± 0.775% across all cultivars and GA levels (Table 4.3.3), with no interaction ($P < 0.500$).

Weed content did not differ ($P < 0.143$) among cultivars, averaging 13.5 ± 7.75% across all cultivars and GA levels (Table 4.3.3), and there was no interaction ($P < 0.500$).

Table 4.8: Botanical composition (%) on the 06/10/2017 of pure subterranean clover with (+) or without (-) GA, applied on the 31/07/2017 at Lincoln University, New Zealand.

Cultivar	Weed content (%)		Subterranean clover content (%)	
	-GA	+GA	-GA	+GA
Antas	5.78	9.1	94.2	90.9
Denmark	10.8	17.7	89.2	82.3
Leura	23.3	19.1	76.7	80.9
Monti	26.2	22.1	73.8	77.9
Narrikup	8.19	5.57	91.8	94.4
Woogenellup	2.36	11.9	97.6	88.1
S.E.M		7.75		

5 DISCUSSION

The overall aim of this research was to identify ways to increase the legume content of pastures in early spring. To do this three separate experiments were undertaken. This section discusses the results before general comments are made in Chapter 6.

5.1 Experiment 1

Experiment 1 over-drilled subterranean clover in autumn. Three different cultivars were used to determine which, if any, could compete with perennial ryegrass.

5.1.1 Dry matter yields.

'Woogenellup' was the most suitable of the three subterranean clover cultivars used in this experiment to increase the early spring legume content of this ryegrass based pasture. Pastures over-drilled with 'Woogenellup' produced more ($P < 0.05$) dry matter than the other treatments (Figure 4.1.4). 'Woogenellup' also grew more subterranean clover than 'Denmark' (Figure 4.1.7), and had higher total clover content than all other pastures (Table 4.1.1). Grass yields did not differ among treatments (Figure 4.1.5), which shows the differences in dry matter yield was the result of increased subterranean clover production. These findings contradict those from Wright (2015), who evaluated 11 subterranean cultivars, including 'Antas', 'Woogenellup' and 'Denmark', in cocksfoot based pastures. Wright (2015) showed 'Antas' had higher ($P < 0.001$) subterranean clover yields than all other cultivars. Wright (2015) also showed plant height, where 'Woogenellup' was the tallest of the three cultivars in her research. This could explain why 'Woogenellup' had greater total dry matter and total clover yields than the other treatments. The taller growth of 'Woogenellup' may have meant it was more competitive for light with perennial ryegrass, which results in greater growth. This is consistent with the comparatively poor growth of 'Denmark'. 'Denmark' has a prostrate growth habit (LUDPRT, 2016), so may struggle to compete with ryegrass in the pasture.

Another reason for the greater production of 'Woogenellup' could have been the large leaf size, particularly when compared with 'Denmark'. Pecetti & Piano (1998) noted that increased leaf size generally led to an increase in the competitiveness of subterranean clover. This could explain the lower production of 'Denmark' (small leaves) in comparison to 'Woogenellup' (large leaves), however it does not explain the poor growth of 'Antas', which is also a large leaved cultivar. Poorer growth of 'Antas' could be attributed to the pH level in the soil. Soil pH was 5.7 in Experiment 1 (Table 3.2). 'Antas' belongs to the *brachycalycinum* sub species. Bolland (1987) noted that cultivars of the *brachycalycinum* sub species are suited to higher pH soils. This could explain why 'Antas' produced more in Experiment 2 (Figure 4.2.5), where pH ranged from 6.6-6.8. 'Woogenellup' and 'Denmark' are both from the *subterraneum* sub spiecies, which is tolerant of the slightly acidic soils found in Experiment 1 (Table 3.1). Alternatively, the poor growth of 'Antas' could have been due to slower leaf appearance (Figure 4.1.3) leading to a slower establishment. 'Antas' produced a leaf every 150 °Cd until it reached secondary leaf appearance (leaf number 5). The long phyllochron for 'Antas' means perennial ryegrass could have out-competed it easier, which may have been the cause of low subterranean clover yields in 'Antas' patures. Subsequently, total dry matter yields in 'Antas' pastures did not differ to the control at Experiment 1 (Figure 4.1.4).

5.1.2 Low overall yields

Of note are the low subterranean clover yields overall in Experiment 1 (Figure 4.1.7) in comparison with other experiments where subterranean clover was grown in a pasture mix. 'Woogenellup' grew the most ($P < 0.05$) subterranean clover (173 kg DM/ha), while 'Antas' and 'Denmark' grew 90 and 61 kg DM/ha, respectively. Mills *et al.,* (2008) showed the clover yields of several dryland pastures over five years in an experiment at Lincoln University. Subterranean clover, grown with cocksfoot, yielded 3.5-4 t DM/ha in three of the five years, and produced at least 1.5 t DM/ha in all years. These low yields meant that there was not increase in clover content, which was one of the main aims of this experiment.

The differences between these yields and the current research is likely to be due to several factors. Firstly, higher than average rainfall (Figure 3.1), particularly in March, April, and July, may have had a negative impact on yield. Subterranean clover has been shown to cope poorly with waterlogged soils. Francis & Devitt (1969) showed waterlogged subterranean clover seedlings produced 26% less herbage compared with controls. The lower yield from water logged soils was attributed to the inhibition of root growth when soils are saturated (Section 2.3.5.3). When seedlings were establishing in March and April, rainfall was almost double the long term average (Figure 3.4.2). This may have inhibited root growth of the seedlings, which could have led to slower establishment. Further research could investigate this factor.

A second factor that may have contributed to low yields was damage from slugs and grass grub. Slugs were a continuous problem throughout the experiment (Section 3.1.5). Grass grub was also discovered, and caused considerable damage to the trial area, completely wiping out all pasture in some areas. The number of established seedlings (Figure 4.1.1) showed that slugs hadn't killed many seedlings by the 21/04/2017. The number of seedlings established was about 82% of the number of seeds sown for 'Antas' and 'Woogenellup' (Table 3.1). About 59% of sown 'Denmark' seeds were established. The lower establishment percentage for 'Denmark' is likely to be due to a smaller seed size (Table 3.1) (Hampton *et al.,* 1987) rather than slug damage. However, failure to control the slugs after they were first discovered meant that slugs continued to damage seedlings after the establishment count was taken. This would have resulted in reduced leaf area in established seedlings, meaning less light interception and slower establishment. Failure to control slugs was probably due to slug bait only being applied to the experimental area rather than the whole paddock. It is likely this enabled slugs to quickly return to the experimental area from adjacent areas.

A third factor that may have contributed to poor subterranean clover growth was a low level of plant available phosphorus (Olsen P). The Olsen P level at Experiment 1 was 13 mg/L (Table 3.1). In comparison, Jordan (2011) showed the optimum (97% maximum yield) Olsen P level for subterranean clover growth to be 45 mg/L. Each of these factors contributed to slow establishment, meaning plants were not fully established by the time cold temperatures and frosts reduced growth in mid-winter (Figure 3.4.1). The slow establishment of the sub clover meant that the pasture needed to be grazed in mid-August to stop the ryegrass from out-competing the clover. Ideally the pasture would have been spelled from early-mid winter, allowing subterranean clover to take advantage of warming temperatures in early spring.

5.1.3 Botanical composition.

The weed content of 'Woogenellup' and 'Antas' was higher ($P < 0.05$) compared with 'Denmark' and the control (Figure 4.1.6). The increase in weeds may have been attributed to a more open sward in some areas, allowing subterranean clover and broadleaf weeds to intercept more light, resulting in greater production. If this is the case, then it could be concluded that 'Woogenellup' and 'Antas' grew well in more open pastures that allowed greater light interception, whilst 'Denmark' grew in denser pastures where weed growth was restricted. Seefeldt & Armstrong (2000) showed the effects of pasture density on the weed component of pastures. Perennial ryegrass was sown at rates of 0, 5, 10, 20, 40, and 80 kg/ha. Increasing rates of pastures resulted in decreased weed yields, which was attributed to greater shading in dense pastures, therefore less light interception.

5.1.4 Establishment.

All cultivars had similar seedling establishment on the 21/04/2017, averaging 85.4/m² (Figure 4.1.1). This is below the optimum establishment recommended by Sheath & Macfarlane (1990), who noted that regenerating subterranean clover pastures should have 150-200 established seedlings/m² in June should maximise spring growth. This would have contributed to the low production of subterranean clover in spring in comparison with previous studies (Section 5.1.2). Based on thousand seed weights (Table 3.1.1), a sowing rate of 10 kg/ha only sows 96-145 seeds/m², meaning that 150-200 seedlings/m² was unachievable at Experiment 1. A sowing rate of 15-20 kg/ha may have increased seedling establishment, however this rate would be too expensive to use on a commercial farm. Slug damage was a contributing factor to the poor establishment of subterranean clover in this experiment (Section 3.1.5, Plate 3.1). The extent of slug damage was not quantified in this experiment. However, Glen *et al.,* (1991) showed slugs reduced seedling establishment of ryegrass/white clover pastures, to the point where white clover content was <1%. Further, Glen *et al.,* (1991) showed slugs preferred grazing on clover as opposed to ryegrass or weeds. This means that establishing subterranean clover seedlings would have been particularly vulnerable to slug attack, resulting in lower subterranean clover and total dry matter yields.

Another factor that contributed to the poor establishment of subterranean clover was the competitiveness of perennial ryegrass. Competition for light and nutrients from perennial ryegrass can make it difficult for slower establishing clover species to survive during the establishment phase. Hurst *et al.,* (2000) used different methods of establishment to maximise clover content of dairy pastures in North Otago. Clover content of 5 month old pastures was reduced from 21% to 12% when perennial ryegrass sowing rates were increased from 3.5 kg/ha to 8 kg/ha. These results showed the negative effect that perennial ryegrass can have on establishing species. This competition would probably be stronger in established perennial ryegrass pastures such as the ones used in Experiment 1 and 2. This competitiveness likely contributed to the below-optimum establishment of subterranean clover in Experiment 1. Hurst *et al.,* (2000) also showed the results of over-drilling ryegrass in autumn into established white clover swards that were sown in November. This method resulted in >60% legume content the following October. This suggests that establishing clover prior then over=drilling ryegrass may be a successful method for increasing the clover content of pastures. Also of note in the experiment by Hurst *et al.,* (2000) is that clover content of pastures sown as ryegrass/clover mixes reached 50% 16 months after sowing with both sowing rates. This meets the optimum clover content of dairy pastures noted by Harris *et al.,* (1997) (Section 2.2.2). These results show that low sowing rates of ryegrass can result in increased clover content of dairy pastures. This could be an option for farmers to increase legume content of pastures rather than over-drilling. However, early spring legume content may still be low due to the

5.1.5 Leaf accumulation and leaf appearance.

Leaf accumulation did not differ between cultivars (Figure 4.1.2), although 'Denmark' did have a shorter average phyllochron than 'Antas' and 'Woogenellup'. This is likely related to leaf size. 'Denmark' is a small leaved cultivar, while 'Antas' and 'Woogenellup' are both large leaved cultivars (LUDPRT, 2016). Thermal time has been shown to be a driver of leaf production (Moot *et al.,* 2003b). The results from this experiment show a smaller thermal time requirement for the production of smaller leaves. A split line regression showed all cultivars commenced secondary leaf production between leaf number five and six (Figure 4.1.3). This is similar to findings by Richardon (2003) and Moot *et al.,* (2003b), who both showed subterranean clover to commence secondary leaf production at main stem leaf number 5. While the leaf stage at which secondary leaf production was the same across cultivars in this experiment, the thermal time accumulation was not. In this experiment secondary leaf production commenced between 828 °Cd ('Denmark') and 1058 °Cd ('Antas'). This is much higher than the 430 °Cd that was noted by Richardon (2003) and Moot *et al.,* (2003b). The differences in thermal time accumulation for secondary leaf appearance between this experiment and previous research may have been due to slower establishment and the removal of cotyledons by grass grub and slug damage (Section 3.1.6). This indicates that secondary leaf production is dependent on leaf stage rather than strictly thermal time accumulation. Again, there was a contrast in phyllochron between the small leaved 'Denmark' and the large leaved 'Antas', both before and after secondary leaf production commenced. Interestingly, 'Woogenellup' had a phyllochron almost identical to 'Denmark' until secondary leaf production began (93 vs. 95 °Cd). However, once secondary leaf production commenced, 'Woogenellup' had a phyllochron similar to 'Antas' (50 vs. 53 °Cd), while the phyllochron for 'Denmark' dropped to 36 °Cd. This may mean that 'Woogenellup' produced small leaves until it reached secondary leaf production, after which it focused on maximising leaf area. This could mean that 'Woogenellup' is faster at establishing a canopy to intercept light compared with other large leaved cultivars such as 'Antas', and could explain the greater subterranean clover yields produced by 'Woogenellup' in this experiment. However, more detailed sampling of leaf area is required to quantify this.

5.2 Experiment 2.

Experiment 2 aimed to increase early spring dry matter yields and clover content of ryegrass based pastures by over-drilling subterranean clover in autumn. Experiment 2 was carried out on a commercial dairy farm in North Otago, with the added aim of investigating whether over-drilling subterranean clover would under the normal dairy farm management.

5.2.1 Dry matter yields.

In contrast to Experiment 1, there was no difference in total dry matter yields among treatments in Experiment 2 (Figure 4.2.2). 'Woogenellup' and 'Antas' had greater total clover yields than 'Denmark' and the control (Figure 4.2.7). However, these differences were cancelled out by a trend for greater grass yields in 'Denmark' and the control (Figure 4.2.3). Higher clover content in the 'Woogenellup' and 'Antas' treatments (Table 4.2.1) may have suppressed grass yields, resulting in no differences in total dry matter yield. Lower total dry matter yields as a result of higher clover content have been reported by Marsh & Laidlaw (1978). They showed pastures with 44% clover had lower ($P < 0.05$) total dry matter yields than pastures with 12% clover. In Experiment 2, grass growth in early spring may have been suppressed by increasing clover yields due to higher light interception, caused by the horizontal aspect of clover leaves. The earlier growth and development of subterranean clover may have placed these leaves above the grass canopy.

Results showed the potential for subterranean clover to increase spring legume content, particularly in areas where white clover is absent. Dry matter yields were similar among treatments, but 'Woogenellup' and 'Antas' had a higher ($P < 0.05$) total clover content (Table 4.2.1). Clover has a higher metaboliseable energy (ME) content than perennial ryegrass, meaning a pasture with higher clover content at the same yield would mean a higher total ME content for pastures with high clover content (Woodward *et al.,* 2003). While the dry matter intake of cows would not have increased in Experiment 2 as a result of over-drilling subterranean clover, an increase in milk solid production may have occurred due to the higher ME value of pastures. Harris *et al.,* (1997) showed milk solid production increased by 0.21 kg MS/cow/day when clover content of pastures was increased from zero to 25% (Section 2.2.2). Clover content in 'Woogenellup' pastures in Experiment 2, as well as Woolshed 5 at Invernia, had clover contents ranging from 20-

26%. In the top and bottom blocks of Woolshed 5, clover content was made up entirely of subterranean clover (Table 4.2.3).

There was a trend for 'Woogenellup' and 'Antas' pastures to have higher weed yields than 'Denmark' and the control pastures (Figure 4.2.6). This was consistent with Experiment 1, where weed yield was higher in 'Woogenellup' and 'Antas' pastures (Figure 4.1.7). This suggests 'Woogenellup' and 'Antas' were more successful in open pastures, which also promote weed growth.

5.2.2 White clover competitiveness.

In Woolshed 5 at Invernia, subterranean clover only grew when white clover was not present (Table 4.2.3). Subterranean clover content was 20 and 25% in the bottom and top blocks of Woolshed 5, while white clover was not present. In contrast, the middle block of Woolshed 5 had 26% white clover, and no subterranean clover. This suggests that the established white clover in the pasture successfully out-competed subterranean clover seedlings when they were trying to establish. This result contradicts those from Airport 6 (Experiment 2), where subterranean and white clovers were shown to successfully grow together (Figure 4.2.7). These differences are likely due to lower white clover content in Experiment 2 (6%), in comparison with the middle block of Woolshed 5 (26%). Scott (2001) noted that competition between white clover and subterranean clover is related to the density of the canopy. When clover content increases, the canopy becomes denser, making it more difficult for a competing clover species to elongate its petiole and position a leaf above the grass canopy. This information could be used to draw conclusions on where it would be appropriate to over-drill subterranean clover. For example, over-drilling could successfully increase the legume content of pastures where white clover content is less than 10% (as seen in Experiment 1, Experiment 2 and the top and bottom blocks of Woolshed 5). However, this method may not be suitable where white clover content is already over 20% in autumn.

5.2.3 Subterranean clover production.

Similair to Experiment 1, 'Woogenellup' produced more subterranean clover than 'Denmark' in Experiment 2 (Figure 4.2.5). However, in contrast to Experiment 1, 'Antas' also grew more subterranean clover than 'Denmark'. The low yield of 'Denmark' and high yield of 'Woogenellup' were consistent across both experiments. A possible explanation is their differences in leaf size and plant height (Section 5.1.1). 'Woogenellup' is taller (Wright, 2015), and has larger leaves than 'Denmark' (LUDPRT, 2016), meaning it can compete more effectively for light. The higher growth of 'Antas' in Experiment 2 may reflect the higher soil pH (6.5) in comparison with Experiment 1 (5.7). 'Antas' belongs to the *bradycalcyninum* sub species, which has been reported to prefer higher pH soils (Section 2.3.2)

5.2.4 Establishment.

There was no difference in the number of established seedlings/m² in Experiment 2 (Figure 4.2.1). This was consistent with results from Experiment 1 (Figure 4.1.1). However, seedling establishment in Experiment 2 was nearly double Experiment 1 (85 vs. 158 seedlings/m²). The seedling establishment in Experiment 2 was greater than the number of seeds/m² that should have been sown based on the thousand seed weights (Table 3.1.1). This suggests that the calibration of the drill used for sowing in Experiment 2 was inaccurate. This also makes it difficult to compare the establishment in Experiment 1 to Experiment 2. Despite greater seedling establishment at Experiment 2, subterranean clover yields were similar for both 'Denmark' and 'Woogenellup' between experiments (Figure 4.1.7, Figure 4.25). 'Antas' produced more seedlings in Experiment 2, however this was likely due to differences in soil pH levels (Section 5.2.3). Subterranean clover yield reached 320 kg DM/ha in Woolshed 5 (top). Unfortunately, no establishment counts were carried out in Woolshed 5, so differences in yields cannot be related back to seedling establishment. Initially this paddock was only meant to run out the drill. However, in hindsight it may have been a more appropriate area to carry out the experiment given the greater yields of subterranean clover that were measured in Woolshed 5 in comparison with the experimental area.

5.3 Experiment 3.

The main aim of Experiment 3 was to increase spring dry matter yields of pure subterranean clover swards by applying gibberellic acid.

5.3.1 Dry matter between GA levels.

Gibberellic acid did not increase dry matter yields at any stage during the experiment (Figures 4.3.3, 4.3.6 and 4.3.9). Gibberellic acid has previously been shown to increase dry matter yields by increasing light interception (van Rossum, 2013). However, in Experiment 3, the sward canopy was already fully closed, meaning that swards were already at maximum light interception. Therefore, light interception was not increased as a result of GA application, meaning dry matter yields were not increased. van Rossum (2013) increased dry matter yields and clover content of dairy pastures by applying GA (Section 2.4.2). The differences in results between Experiment 3 and that experiment probably resulted from the pure clover sward in Experiment 3. In a mixed pasture, light interception can be increased in clover by elongating its petiole, allowing the plant to promote leaves to the top of the canopy, reducing competition from grass species. In a pure sward, assuming the canopy is fully closed, light interception cannot be greatly increased by petiole elongation, as the entire canopy will move upwards, without increasing leaf area.

5.3.2 Plant height between GA levels.

Plant height was increased by GA application 44 days after application (DAA) (Figure 4.3.1). This was because GA degrades DELLA repressor proteins, allowing for increased stem and leaf elongation (Section 2.4.2). However, this increase was no longer apparent 67 DAA, when there was no difference in plant height (Figure 4.3.2). Although there was no longer a difference in plant height at the second measurement, there was no depression in plant height or dry matter yields. Delayed depressions in yields were reported by McGrath & Murphy (1976), who noted a depression of 290 kg DM/ha following an initial increase in yield. These depressions were related to excessive rates of GA being applied. Matthew *et al.,* (2009) reviewed the use of GA for increasing pasture yields. They noted that delayed depressions in yield were only apparent when GA was applied at over 75 g/ha. In Experiment 3, GA was applied at 8 g/ha, so it is unlikely that there was a depression in plant height or yield as a result of GA application. A further measurement date (for example, 90 DAA) would have quantified this, however this wasn't possible due to time constraints. While the initial increase in plant height did not result in increased dry matter yields in this experiment, it is possible that it may have increased the yield of subterranean clover grown in a mixed pasture by increasing plant height and light interception, and represents an area for further research.

5.3.3 Dry matter between cultivars.

The results from this experiment show that larger leaved cultivars produce greater herbage yields, and therefore would likely be more effective at increasing spring legume content and yield if they can be successfully established. Experiment 3 showed differences in the subterranean clover dry matter yield among cultivars at the second and third cuts (Figure 4.3.7 & Figure 4.3.10), although there was no difference among cultivars at the first cut (Figure 4.3.4). This is probably related to leaf size, which was particulary apparent at the third cut (Figure 4.3.10). At the third cut, there was a strong indication for the three large leaved cultivars 'Antas', 'Woogenellup' and 'Narrikup' to have greater dry matter yields than other cultivars. At the second cut (Figure 4.3.7), 'Woogenellup' and 'Narrikup' had greater subterranean clover yields than smaller leaved cultivars. 'Antas' yields were similar to small leaved cultivars, however this was likely related to a high weed content in 'Antas' swards at the second cut (Table 4.3.2). Results from Experiment 3 were similar to results from Caradus & Chapman (1998), who showed larger leaved white clover cultivars produced greater yields than small leaved cultivars. Caradus *et al.,* (1996) also showed a negative relationship between white clover leaf size and stolon density. Although the differences in growth among cultivars did not increase total dry matter yields at Experiment 3, the greater yields of larger leaved cultivars is important for the overall aim of this research (increasing spring legume content of dairy pastures).

5.3.4 Growth rate.

Subterranean clover grew at 6.36 kg DM/ha/°Cd, or 52 kg DM/ha/day, between 16/08/2017 and 06/10/2017. These growth rates are slightly lower than the 70 kg DM/ha/day for cocksfoot/subterranean clover pastures in spring reported by Mills *et al.,* (2008). However, the growth rates mentioned by Mills *et al.,* (2008) were based on long term mean temperatures in October. The growth rates in Experiment 3 were from mid-August to the beginning of October, when temperatures are colder (Figure 3.4.1), therefore lower growth rates could be expected. These growth rates were considerably higher than white clover growth rates shown by Brown *et al.,* (2006). They showed white clover, as part of a cocksfoot based pasture, to grow 10-20 kg DM/ha/day in October. Subterranean clover grew up to 3400 kg DM/ha ('Narrikup') from mid-August to the beginning of October. This demonstrates the potential subterranean clover has for increasing the spring legume content of pastures.

6 GENERAL DISCUSSION AND CONCLUSIONS

6.1 Over-drilling subterranean clover.

Experiments 1 and 2 showed that over-drilling subterranean clover into established perennial ryegrass based pastures can increase dry matter yields and clover content in early spring. However, subterranean clover growth was visually patchy at both experiments, and yields were low. This raises the question of whether over-drilling subterranean clover was profitable in terms of increased revenue vs costs.

6.1.1 Profitability

For successful farmer uptake of any new farming practice, it needs to be shown to be profitable. Yield increases need to be shown to be sufficient to generate a substantial increase in animal production. The subsequent increase in revenue needs to cover costs (e.g. sowing costs), and also generate extra profit to make the concept worthwhile. Table 6.1 shows the theoretical increase in revenue and costs associated with over-drilling subterranean clover.

Table 6.1: Theoretical revenue and costs for over-drilling subterranean clover into dairy pastures. Assumptions are shown below

	Sub clover yield (kg DM/ha)		
	189	320	1000
Revenue	$	$	$
Milk solids increase/ha ($6/kg MS)	90.7	153	480
Nitrogen fixation/ha	5.48	9.28	29
	96.2	**162**	**509**
Less costs			
Seed cost	100	100	100
Labour + machinery	40	40	40
	140	**140**	**140**
Profit/loss per ha ($)	**-43.7**	**22.8**	**369**

- Milk solid response = 80 g MS/kg DM (Dalley *et al.,* 2005)
- Nitrogen fixation = $29/ t DM (Section 2.2.3)
- Seed cost = $10/kg (Luisetti seeds, 2017)

The dry matter yields in Table 6.1 are from the 'Woogenellup' yields in Experiment 2 (189 kg DM/ha), the greatest yield in Woolshed 5 (320 kg DM/ha), as well as a proposed goal of 1000 kg DM/ha. The profit shown in Table 6.1 is subject to variation, mostly from dry matter yields, milk solid response and milk solid payout. The maximum subterranean clover yield measured in Experiment 1 & 2 (320 kg DM/ha) would result in a profit of $22.80/ha. This profit may be too small for it to be worthwhile for farmers to over-drill sub clover. If the average subterranean clover yield of 'Woogenellup' from Experiment 2 (189 kg DM/ha) is used for the same calculation, the result is a loss of $44/ha. In contrast, if subterranean clover produced 1000 kg DM/ha, it would generate $369/ha. If 20% of the average NZ dairy farm (144 ha) produced an extra 1000 kg DM/ha from over-drilling subterranean clover, this would result in $10,627 of extra profit. Given the variation in the calculations (e.g. milk payout, production response to extra dry matter), producing an extra 500-1000 kg DM/ha from over-drilling subterranean clover should consistently be profitable. Further research needs to be carried out to see if it is possible to consistently produce an extra 500-1000 kg DM/ha from annual clovers.

6.1.2 Leaf size

In both experiments, 'Woogenellup' and 'Antas' were superior to 'Denmark' in terms of dry matter production, which appeared to be related to leaf size. This was also apparent in Experiment 3, where the large leaved cultivars 'Narrikup', 'Antas', and 'Woogenellup' produced greater dry matter yields than small leaved cultivars. This suggests that to achieve a target of 1000 kg DM/ha of subterranean clover, large leaved cultivars would be most appropriate for use in perennial ryegrass pastures. This inference can be supported by the use of large leafed white clover cultivars in dairy pastures. These have greater ability to elongate their petioles and put leaflets above the ryegrass canopy, therefore intercepting more light than small leafed clovers.

6.1.3 Does over-drilling work?

Based on this research, over-drilling was not proven to be a reliable and profitable method for increasing spring dry matter yields and clover content. Both experiments were damaged by extremely high rainfall in July (Section 3.4). However the concept of using subterranean clover to increase spring dry matter yields and clover content was shown to be an area that needs to receive further research before being accepted or rejected.

6.1.4 Further research

Further research needs to be carried out to investigate if producing 500-1000 kg of extra DM from adding subterranean clover to dairy pastures is realistic. Higher than average rainfall (Section 3.4) and pest damage (Section 3.1.7) are likely reasons for the low subterranean clover yields in Experiments 1 & 2. It is likely that subterranean clover yields could have been greater if experimental sites were treated with slug bait prior to sowing, and soils were not waterlogged. If this experiment was replicated, the choice of a more suitable experimental area may result in greater subterranean yields. A sowing rate of 15 kg/ha, using a large leaved cultivar such as 'Woogenellup', should allow sufficient seedling establishment. Pastures with no insect pressure, particularly from slugs, grass grub and clover root weevil, with low white clover content at the time of sowing would create a favourable environment for subterranean clover establishment. Pastures should also be relatively open to allow maximum light interception to establishing seedlings. Irrigation would reduce the risk of establishing seedlings being exposed to water stress. Spray irrigation (e.g. pivots, fixed grid etc.) would be preferable rather than flood irrigation, as this can simulate a temporary waterlogging effect which may inhibit root growth of establishing seedlings. Soils should be free draining, with a pH of >6.0 and Olsen P of 35+. Adding subterranean clover into a seed mix and sowing conventionally could also be investigated, as this could reduce competition from perennial ryegrass and also reduce sowing costs.

Another area for further research is the use of different annual clovers, particularly balansa clover. Balansa clover is a winter annual, with some similarities in its life cycle to subterranean clover (Monks, 2009). However, balansa clover is tolerant of waterlogged or poorly drained soils which may make it more suitable for moist dairy pastures.

6.2 Experiment 3 - Gibberellic acid on pure subterranean clover.

6.2.1 Dry matter yields with GA

Applying gibberellic acid to pure subterranean clover cultivars did not increase spring dry matter yields. This shows that GA is unlikely to be a useful method for increasing spring production of subterranean clover in a pure sward. Previous research showed GA to increase the legume content of spring ryegrass/white clover pastures (van Rossum, 2013). If subterranean clover was added into a dairy pasture, either via over-drilling or conventionally as part of a seed mix, there is potential for GA to increase the production of subterranean clover by increasing plant height and therefore light interception. However, this may not be profitable for a farmer to apply GA. Further, GA would need to be applied in early August, which coincides with calving on most dairy farms. During calving, resources (such as labour and machinery) are often stretched, meaning applying GA may not be worthwhile for a farmer even if it could make pastures more profitable.

6.2.2 Cultivar differences

There were large differences in dry matter yields between cultivars in Experiment 3. Large leaved cultivars such as 'Narrikup', 'Woogenellup' and 'Antas' produced yields of 3.5-4 t DM/ha by the beginning of October. This information is helpful when choosing cultivars, particularly in when herbage production is the primary aim, rather than seed set and regeneration, for subterranean clover.

6.3 Conclusions

- Experiments 1 & 2 showed that there is potential for subterranean clover to become a useful tool for increasing early spring dry matter yields and legume content of moist ryegrass-based pastures.
- However, based on the yields from this research, over-drilling subterranean clover was not sufficiently profitable to conclude that farmers should be using this method. Further research is required to refine this concept before recommending it to farmers.
- Gibberellic acid was proven not to increase spring dry matter yields of pure subterranean clover. However, plant height was increased, meaning GA may increase subterranean clover yields in a mixed sward. Further research could be carried out to investigate this.
- Large leaved cultivars were shown to produce more dry matter than small leaved cultivars across all three experiments. This is helpful for farmers whose main aim is to maximize herbage production.

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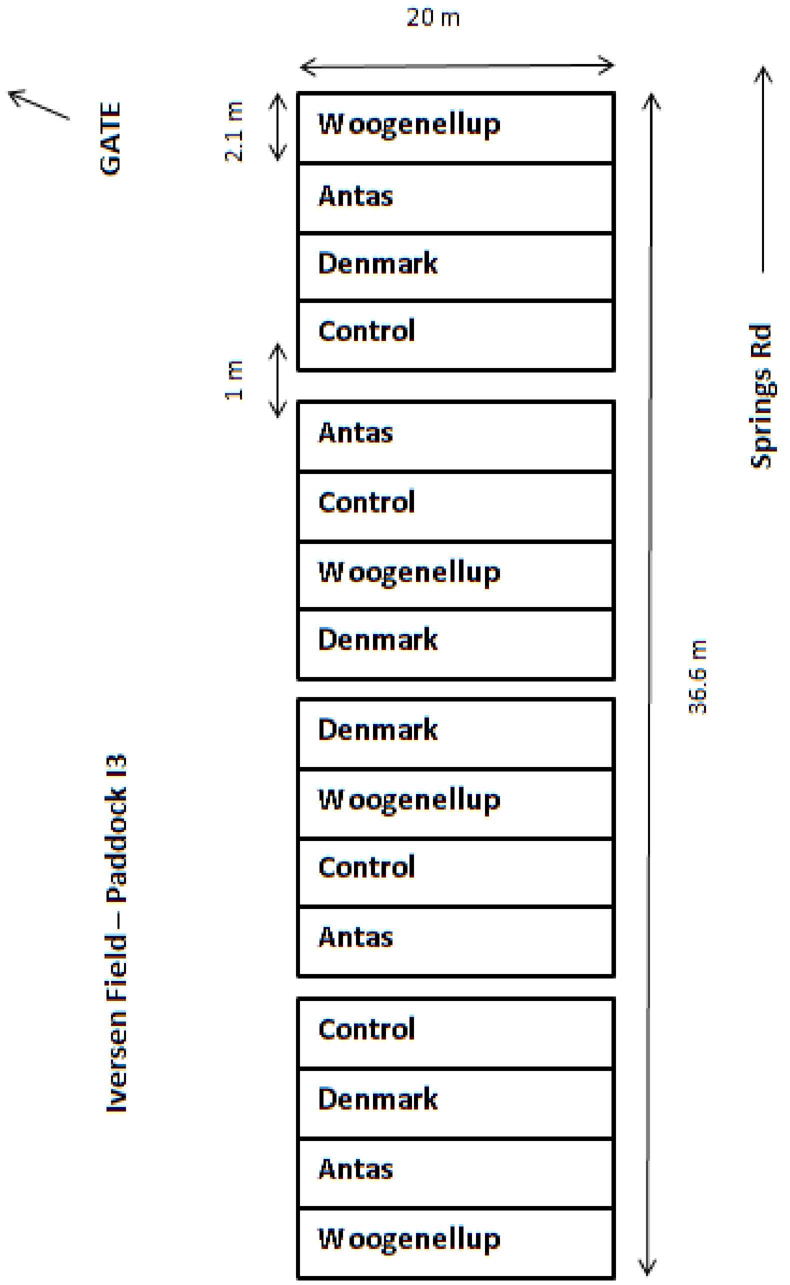
APPENDICES

Appendix 1: Experimental design for Experiment 1 at Iversen Field, Lincoln University, New Zealand.



Appendix 2: Experimental design for Experiment 2 at Invernia, North Otago, New Zealand.